MEDICAL PROTOZOOLOGY AND HELMINTHOLOGY

(REVISED 1965)

U. S. NAVAL MEDICAL SCHOOL
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

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PREFACE

This manual was prepared and edited and has been continuously revised by the staff of the United States Naval Medical School. It is intended primarily as an instructional guide for clinical laboratory students of the Navy, and as a simplified ready reference for both physicians and technicians in the field of Medical Protozoology and Helminthology.

Many sources have been consulted in the preparation of this manual to accomplish the desired objective. Considerable emphasis has been placed upon illustrations and diagrams to facilitate an understanding of the subject matter. The procedures and techniques herein outlined for detection and identification are proven methods currently in use in the laboratories of the United States Naval Medical School, National Naval Medical Center, Bethesda, Maryland.

For additional information on detailed morphology, epidemiology, symptomatology, etc., the student should consult recognized texts in the fields of parasitology and tropical medicine.

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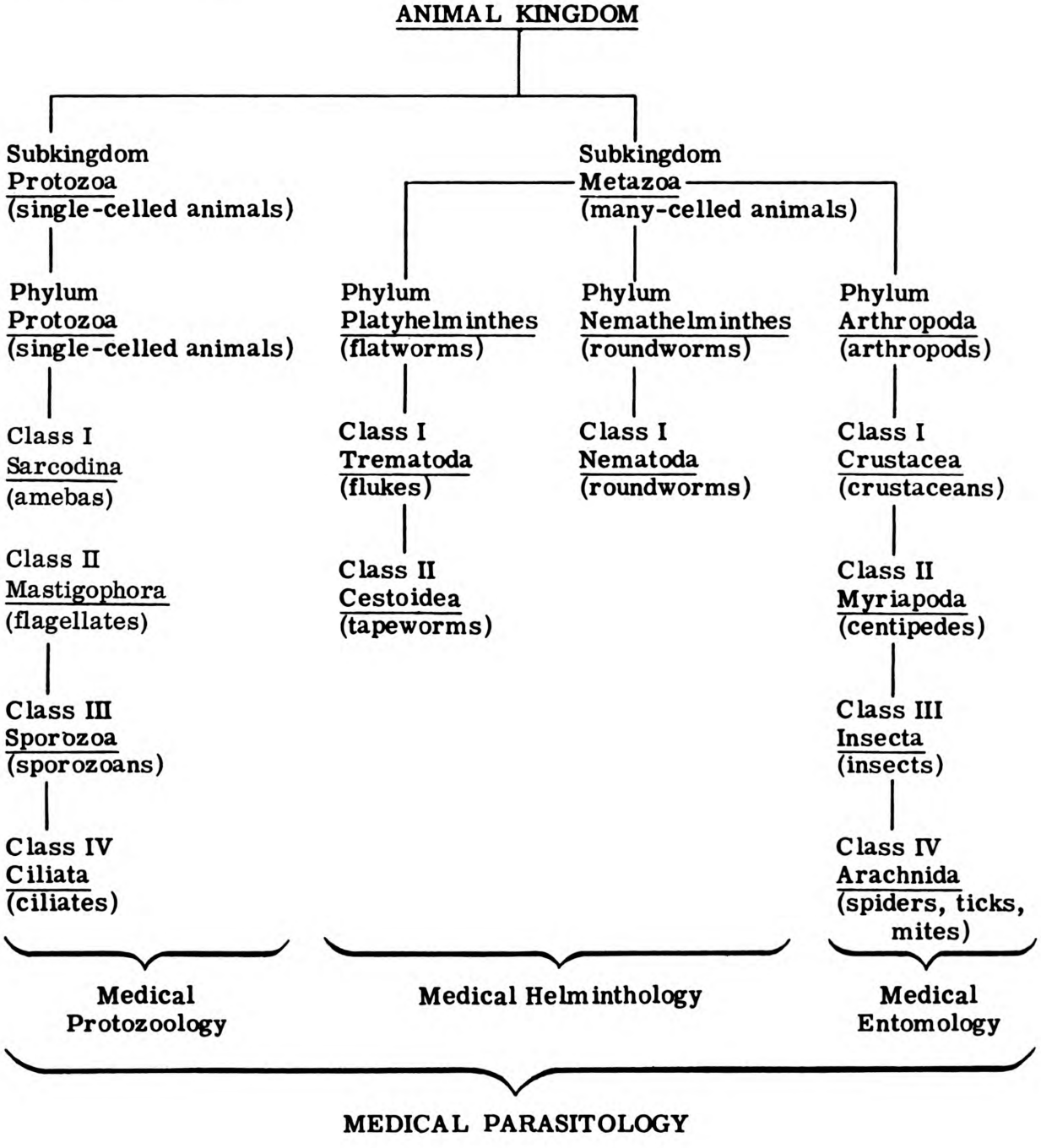
INTRODUCTION

Parasitology is the science of parasitism. Parasitism is the relation between parasites and their hosts. A parasite is an organism that lives on, or within, and is metabolically dependent on some other living organism. We are concerned only with parasitism in animals with emphasis placed on those found in, or of greater importance to man.



DEFINITIONS

Parasitism - an association between two specifically distinct organisms in which one, the parasite, is metabolically dependent on the other, the host.

Parasite - an animal or plant that lives in or on another organism, the host, from which it requires something for its existence and development and in which it sometimes causes disease.

Endoparasite - a parasite that lives within the body of the host.

Ectoparasite - a parasite that lives upon the body of the host.

Erratic parasite - a parasite that wanders into an organ in which it does not usually live.

Facultative parasite - an organism that is capable of living either free or as a parasite.

Incidental parasite - a parasite that establishes itself in a host in which it does not usually live.

Obligatory parasite - a parasite which depends for its existence upon its host.

Periodic parasite - a parasite that makes short visits to its host to obtain nourishment or other benefits.

Permanent parasite - an organism that is parasitic throughout its entire life cycle.

Pseudoparasite - an object that is mistaken for a parasite.

Temporary parasite - a parasite that is free-living during a part of its life cycle.

Host - an organism which harbors a parasite.

Definitive host - the host which harbors the adult or sexual stages of the parasite.

Vector host - the host usually responsible for infection of the vertebrate host.

Intermediate host - the host which harbors the larval or asexual stages of the parasite and one in which there may be effective parasite multiplication.

First intermediate host - the first host parasitized by the immature stages of the parasite. Usually this host is an invertebrate.

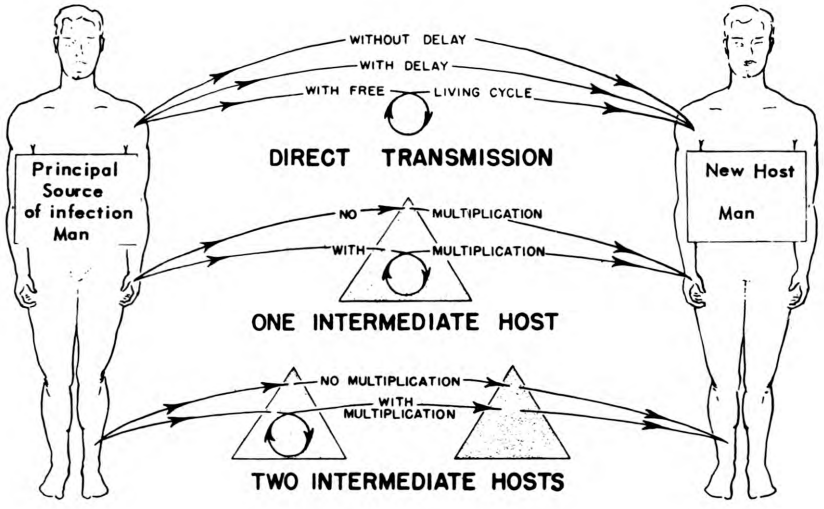
Second intermediate host - a host which harbors an immature stage of a parasite after it has left the first intermediate host. This host may be a vertebrate or an invertebrate.

Reservoir host - a host, usually a vertebrate, other than the host of interest, which is able to take its place in the life cycle of the parasite and may serve as a source of infection.

NOMENCLATURE

In the classification of animal parasites, numerous subdivisions of the main groups are necessary. The names applied follow the laws laid down in the "International Rules of Zoological Nomenclature* ". The parasites with which we are concerned belong to three phyla of the animal kingdom: Protozoa, Platyhelminthes, and Nemathelminthes. Each phylum is divided first into classes and these into orders, families, genera, and species. Scientific names must be Latin or Latinized; family names are formed by adding -idae to the stem of the name of the type genus; generic names should consist of a single word written with a capital initial letter and italicized; the specific name should always begin with a small letter. The author of a scientific name is the person who first publishes the name with a definition or description of the organism given. The names of the genera and species are underlined in writing them. This serves as an instruction to print them in italics.

*For an explanation of the principles regarding nomenclature refer to: Faust, E. C., and Russell, P. F., 1957. Clinical Parasitology, Lea & Febiger, p. 29, or Faust, E. C., 1955, Animal Agents and Vectors of Human Disease, Lea & Febiger, p. 24.



DIRECT TRANSMISSION
(Intermediate host not required)

Without delay—

parasite immediately infective for man:
- Entamoeba histolytica
- Giardia lamblia
- Trichomonas vaginalis
- Balantidium coli
- Plasmodium species (under special circumstances)*
- Trypanosoma species (under special circumstances)
- Leishmania species (under special circumstances)
- Hymenolepis nana
- Enterobius vermicularis
- Sarcoptes scabiei
- Pediculus humanus
- Phthirus pubis

With delay—

parasite must develop to infective stage:
- Enterobius vermicularis
- Ascaris lumbricoides
- Trichuris trichiura
- Ancylostoma species
- Necator americanus
- Strongyloides stercoralis
- Trichinella spiralis

With free-living cycle—

parasite must develop to infective stage:
- Strongyloides stercoralis (under special circumstances)

ONE INTERMEDIATE HOST
(Obligatory)

No multiplication but parasite undergoes development in intermediate host:
- Wuchereria species
- Onchocerca volvulus
- Loa loa
- Dracunculus medinensis
- Taenia species
- Dipylidium caninum
- Hymenolepis diminuta
- Hymenolepis nana (under special circumstances)

With multiplication and development in intermediate host:
- Schistosoma species
- Trypanosoma species
- Leishmania species
- Plasmodium species*
- Echinococcus granulosus*

TWO INTERMEDIATE HOSTS
(Obligatory)

No multiplication but parasite undergoes development in both intermediate hosts:
- Diphyllobothrium latum

With multiplication in first intermediate host—development in both:
- Clonorchis sinensis and other liver flukes
- Fasciolopsis buski and other intestinal flukes
- Paragonimus westermani

*In these species man is the intermediate host and some other animal the definitive host. The life cycles, however, fall easily into the above scheme.

Fig. 1 - Principles and means of parasite transmission

MORPHOLOGY AND BIOLOGY

The Protozoa are unicellular animals. Like each cell of the Metazoa (many-celled animals), their single cell consists of cytoplasm and a nucleus or nuclei. The cytoplasm is differentiated into an outer layer, the ectoplasm, and an inner mass, the endoplasm.

The ectoplasm is a dense, resilient structure. It performs the functions of the skin (protection), the limbs (locomotion), the mouth (ingestion of food), and the excretory organs of the higher animals. The most obvious of these functions is locomotion. It is accomplished by means of what has been termed ectoplasmic organelles: in the ameba, ectoplasmic protrusions - pseudopodia; in the flagellates, long, thread-like filaments - flagella; and in the ciliates, short, hair-like filaments - cilia. These organelles also serve to procure food. Some species of protozoa encyst; the ectoplasm then is modified into the firmer, more resistant cyst wall.

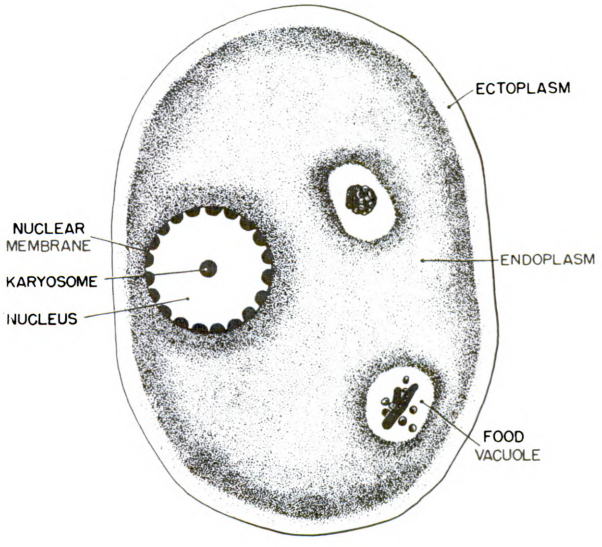
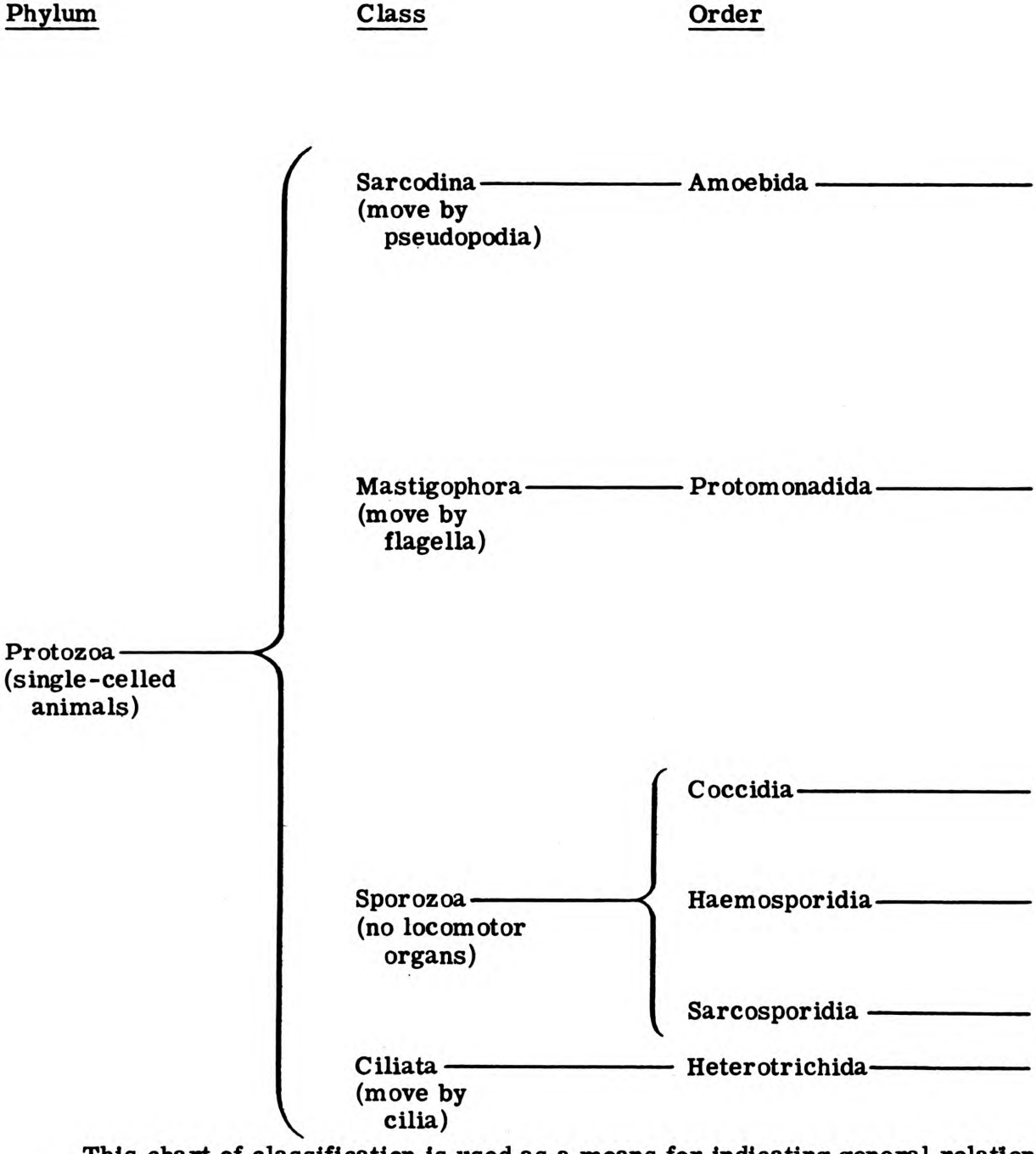


Fig. 2 - A protozoan

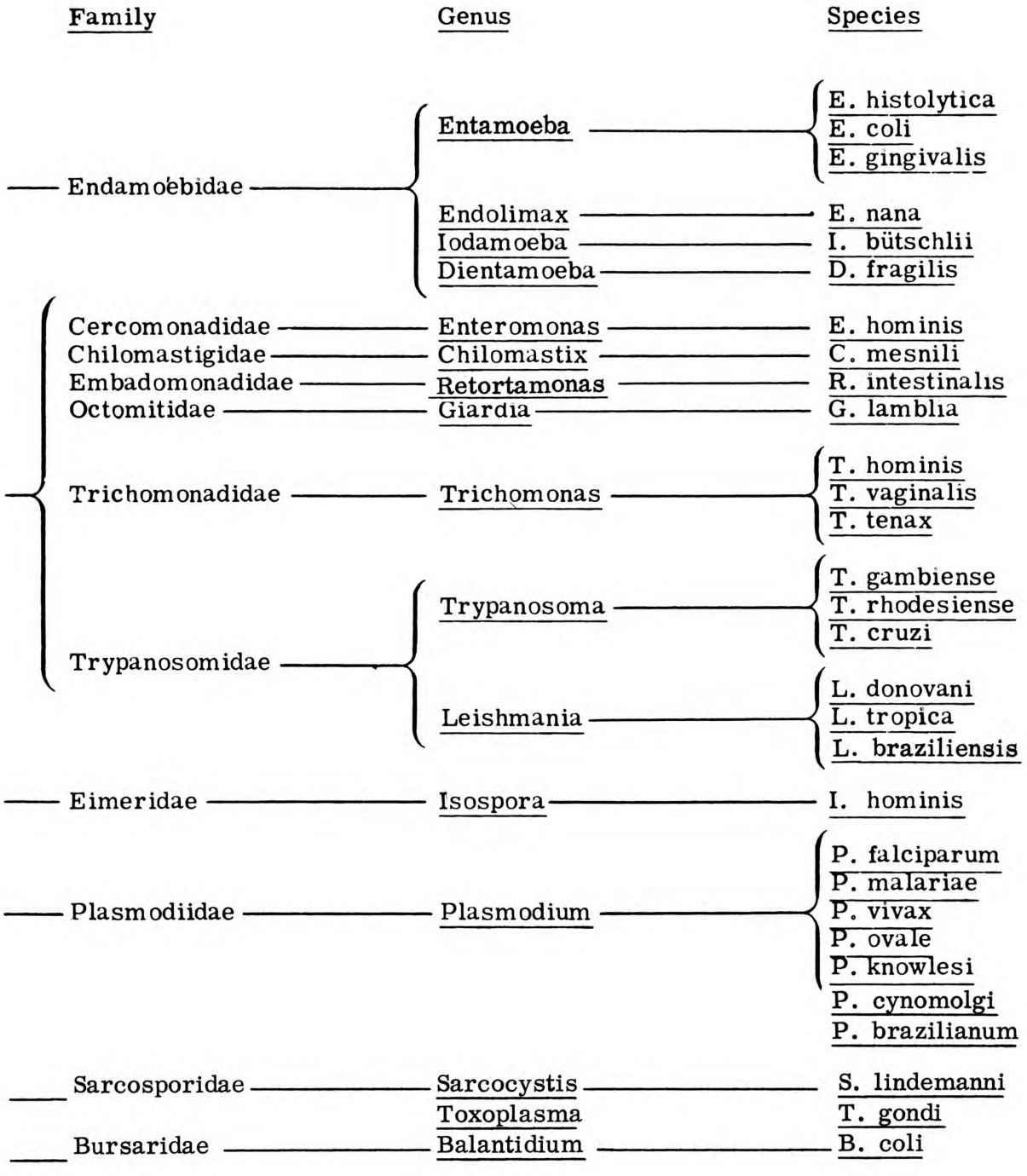
The endoplasm is a thinner substance, flowing like water or a syrupy solution within the ectoplasm. It contains various inclusions, i.e., mitochondria, Gelgi bodies, etc. It is the "viscera" concerned mainly with nutrition. It usually contains ingested material in various stages of digestion, such as bacteria, vegetable cells, tissue cells, and starch granules. Some of these may be within vacuoles, so-called food vacuoles. In some protozoa there are also contractile and hydrostatic vacuoles in the endoplasm which have to do with the elimination of waste products and with fluid balance.

The nucleus is concerned chiefly with reproduction and control of cell functions, Its structure varies greatly. In some species it appears to be merely a mass of chromatin, while in others it has a definite and complex organization. These variations are used in many instances in the identification of protozoa. The details of nuclear morphology and methods of reproduction will be discussed in the description of the various genera and species.

CLASSIFICATION



This chart of classification is used as a means for indicating general relationships among the protozoa studied. It should be realized that a given taxonomic scheme is not necessarily accepted by all authors or workers in this field of study.


Family
Genus
Species
Endamoebidae
Entamoeba
E. histolytica
E. coli
E. gingivalis
Endolimax
E. nana
Iodamoeba
I. bütschlii
Dientamoeba
D. fragilis
Cercomonadidae
Enteromonas
E. hominis
Chilomastigidae
Chilomastix
C. mesnili
Embadomonadidae
Retortamonas
R. intestinalis
Octomitidae
Giardia
G. lamblia
Trichomonadidae
Trichomonas
T. hominis
T. vaginalis
T. tenax
Trypanosomidae
Trypanosoma
T. gambiense
T. rhodesiense
T. cruzi
Leishmania
L. donovani
L. tropica
L. braziliensis
Eimeridae
Isospora
I. hominis
Plasmodiidae
Plasmodium
P. falciparum
P. malariae
P. vivax
P. ovale
P. knowlesi
P. cynomolgi
P. brazilianum
Sarcosporidae
Sarcocystis
S. lindemanni
Toxoplasma
T. gondi
Bursaridae
Balantidium
B. coli

Sarcodina (Flesh-like) or Rhizopoda (Root-footed) Fig. 3

This class contains one family of medical importance, commonly called the amebas, which move by means of pseudopodia - root-like extensions of the cytoplasm. These pseudopodia also serve as structures for obtaining food. Ectoplasm, or both ectoplasm and endoplasm, may take part in making up the pseudopodia. These organelles vary in shape and characteristic activity in the different species.

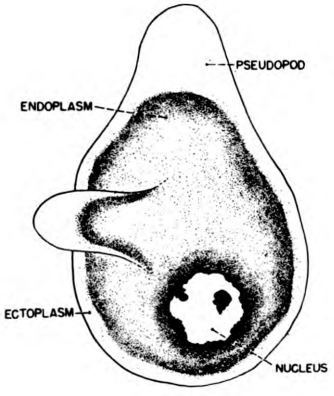


Fig. 3 - An ameba

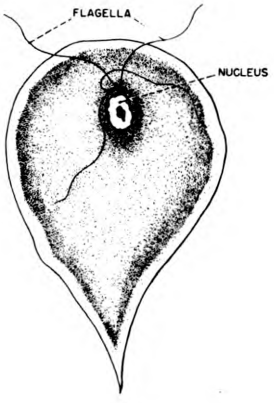


Fig. 4 - A flagellate

Mastigophora (Whip-bearing) Fig. 4

This class contains several families of human protozoa, all belonging to the same order. Several important diseases, such as African sleeping sickness and kala-azar are caused by representatives of this group. The motile forms of this class have thread- or whip-like processes, flagella, for the purpose of locomotion

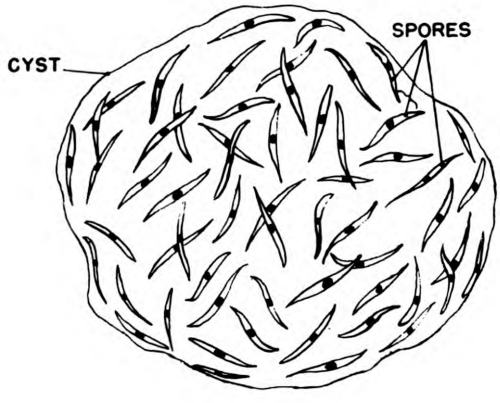


Fig. 5 - A sporozoan

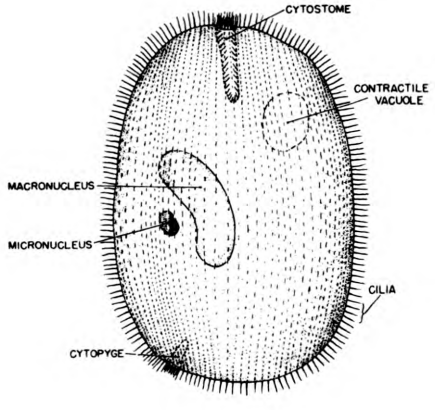


Fig. 6 - A ciliate

and obtaining food. Some species have an undulating membrane in addition to the flagellum.

Sporozoa (Spore-animal) Fig. 5.

This class is identified by the method of reproduction and by the absence of locomotor organelles. Reproduction may be asexual (schizogony) or sexual - after union of male and female gametes and the production of spores (sporogony). All the Sporozoa of man are parasitic within cells, tissues, or body cavities. This class includes the important malaria parasites.

Ciliata

Only one species of medical importance is included in this class. These animals are characterized by a covering of hair-like cilia which serve for locomotion and for direction of food particles into the mouth; the organelles may be evenly distributed over the entire animal or more prominent in certain regions. The body is enveloped in a cuticle which may have only one opening, the cytostome (mouth), or a second opening, the cytopyge (anus). The ciliates usually have both a large macronucleus and a small micronucleus.

By comparing the two tables on pages 7 and 10, it is immediately apparent that not all of the human protozoa cause disease. Some are pathogenic, while others do not seem to be. Since the nonpathogenic forms may be confused with the specific etiologic agents of certain diseases, Medical Protozoology must include a study of all the protozoa to be found in man.

The human protozoa of major importance readily fall into two groups based upon their habitat in the body - the blood protozoa and the intestinal protozoa; a third group of less importance is made up of those inhabiting other parts of the body. These groups provide a better classification for clinical and laboratory study than their zoological relationships. For this reason, the material pertaining to Medical Protozoology will be presented under the headings of blood protozoa and intestinal protozoa; the other protozoa are included with related organisms of the latter group.

PROTOZOA CAUSING SPECIFIC HUMAN DISEASES

Parasites (Diseases)	Vertebrate Hosts	Vector Hosts	Important Reservoir Hosts	Transmission to Man
Entamoeba histolytica (Amebiasis)	Man	None	None	By ingestion (mature cyst)
Balantidium coli (Balantidiasis)	Hogs, Man	None	Hogs	By ingestion (mature cyst)
Giardia lamblia (Giardiasis)	Man	None	None	By ingestion (mature cyst)
Trichomonas vaginalis (Trichomonad vaginitis)	Man	None	None	By contact (flagellate)
Trypanosoma gambiense Trypanosoma rhodesiense (African sleeping sickness)	Man, Animals	Tsetse flies (Glossina species)	Various animals	By inoculation (bite of fly)
Trypanosoma cruzi (Chagas' disease)	Animals, Man	Reduviid bugs, (Triatoma species)	Armadillos, Opossums Man	By contamination of bite by infective feces of bug
Leishmania donovani (Kala-azar) Leishmania tropica (Oriental sore) Leishmania braziliensis* (Espundia)	Man, Dogs	Sand flies (Phlebotomus species)	Dogs	By inoculation (bite of fly; direct transmission possible)
Plasmodium vivax Plasmodium falciparum Plasmodium malariae Plasmodium ovale Plasmodium cynomolgi (Malaria)	Man, monkeys (for P. cynomolgi)	Female anopheline mosquitoes	None	By inoculation (bite of mosquito; also by transfer of infected blood)

*Dogs and other animals have been implicated in the life cycles of Leishmania donovani and L. tropica but the part played by hosts other than man in the case of L. braziliensis is yet questionable. Naturally infected dogs have been found in South America.

INTESTINAL PROTOZOA

DEFINITIONS

Trophozoite - the motile form which feeds, multiplies, and maintains the colony in the host. (These are also called "vegetative forms" and "trophic forms." The term "trophozoite," although strictly belonging to the Sporozoa, is more convenient.)

Cyst - the immotile form protected by a cyst wall and designed for transmission to new hosts.

Encystation - the transformation of a trophozoite into a cyst.

Precystic form - a rounded trophozoite just before encystation.

Excystation - hatching of the cyst with the liberation of a motile metacystic trophozoite.

Chromatin - the portion of the nucleus which is readily stained.

Chromatoid - the material staining like chromatin, found in the cytoplasm and not part of the nucleus.

Volutin - a chromatoid substance occurring as granules in the cytoplasm.

Karyosome - nucleolus, a dark-staining body in the chromatin network of the nucleus.

Blepharoplast - a small dark-staining mass forming the base of a flagellum, acting as a center for movement of the organism.

CLASSIFICATION

The following protozoa have been found to inhabit the human intestinal tract:

Five species of SARCODINA
- Entamoeba histolytica
- Entamoeba coli
- Endolimax nana
- Iodamoeba bütschlii
- Dientamoeba fragilis

Five species of MASTIGOPHORA
- Giardia lamblia
- Chilomastix mesnili
- Trichomonas hominis
- Retortamonas intestinalis
- Enteromonas hominis

One species of CILIATA
- Balantidium coli

Two species of SPOROZOA
- Isospora hominis
- Isospora belli

*For taxonomic relationships of these protozoa, see pages 6 and 7.

PATHOGENICITY

Entamoeba histolytica is by far the most pathogenic of the intestinal protozoa. Two strains, i.e. the large and small types, are recognized. The former apparently is the cause of amebic dysentery and of various less severe intestinal disturbances as well as of amebic abscess of the liver and other organs. The other amebas are usually considered harmless parasites, living in the lumen of the bowel. It is possible that Dientamoeba fragilis may at times be pathogenic, but sufficient evidence for its incrimination is not available. Giardia lamblia is the only flagellate which has been definitely shown to cause intestinal disturbances. The ciliate, Balantidium coli, may in some instances cause ulcerative colitis and chronic dysentery.

Clinicians might be justified in limiting their interest to the three protozoa which are known to cause disease. Such limitation is not possible, however, in the clinical laboratory, mainly on account of Entamoeba histolytica. This ameba may resemble not only the other four species of ameba but also the flagellates. It is, therefore, necessary to know and learn to recognize some distinguishing characteristics of each of the intestinal amebas and flagellates in order to differentiate them from E. histolytica. This task is undoubtedly the most difficult in the laboratory diagnosis of parasitic diseases, and few workers find time and opportunity to master it. Differentiation of the smaller and larger strains of E. histolytica may be important in certain cases of infection. Reliable differentiation depends upon accurate observation and measurement of trophozoites and cysts.

LIFE CYCLES

In order to understand the appearance in feces of the various forms of the intestinal protozoa, it is necessary to know the essential features of their life cycles. The life cycle of Entamoeba histolytica will be given first and in some detail; then the life cycles of the others may be understood by merely referring to similarities and differences.

Entamoeba histolytica (Fig. 7)

The preferred habitat of E. histolytica is the colon. Here it usually lives in the lumen, causing its host no annoyance. Occasionally it invades the bowel wall, producing ulcerative lesions and dysentery. Sometimes the invading trophozoites are transported in the blood vessels to the liver and occasionally to other organs where they multiply and cause abscesses. E. histolytica feeds on blood and tissue elements when available; otherwise it apparently exists on undigested food particles in the fecal stream. It multiplies by binary fission and thereby maintains a colony against a daily loss of millions in the feces. Under certain conditions some of the lumen trophozoites (not those within the host tissues) become encysted. The

LIFE CYCLE OF ENTAMOEBA HISTOLYTICA

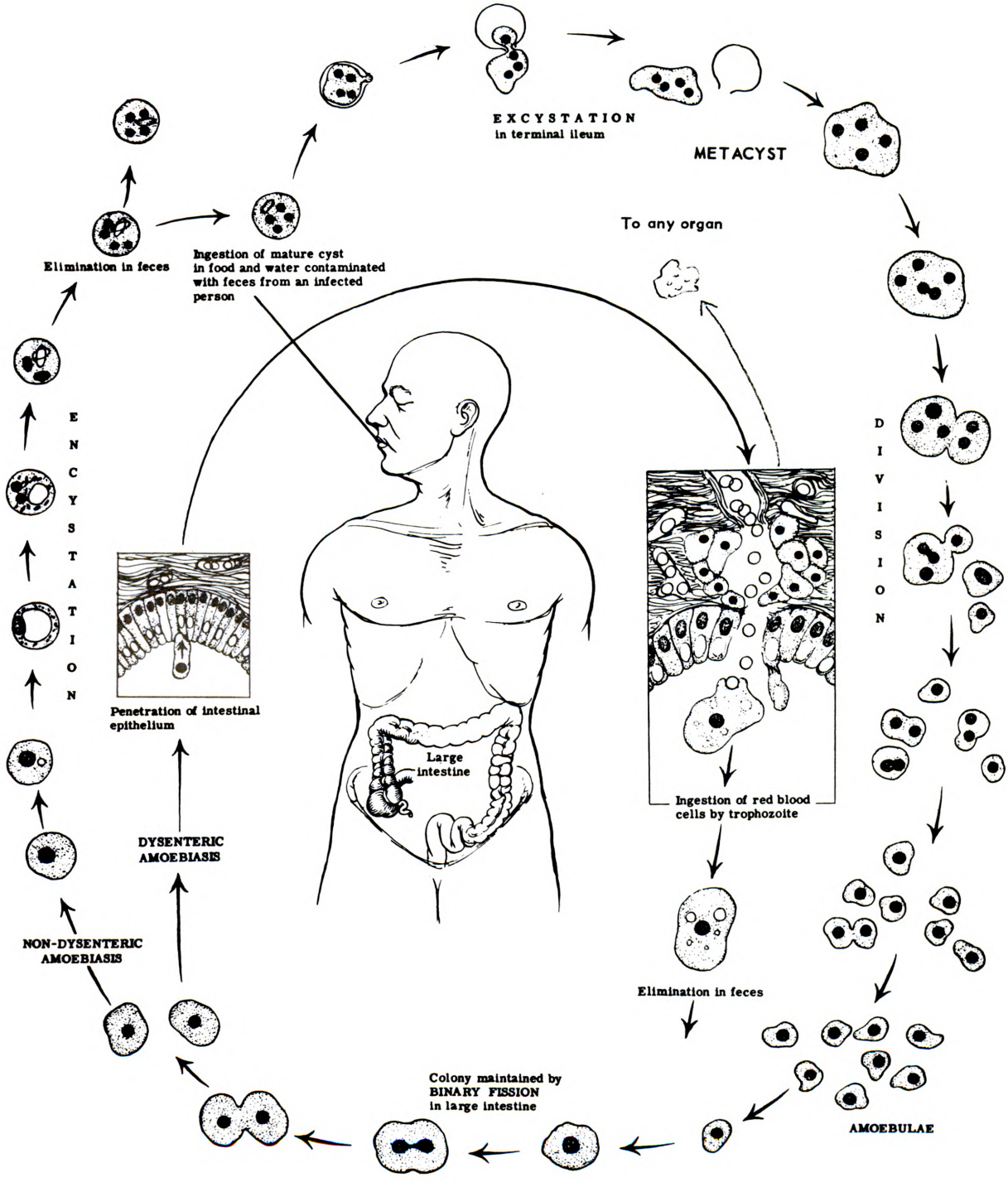


Fig. 7 - Life Cycle of Entamoeba histolytica

encysting trophozoite first loses its motility, stops feeding, and rounds up. The ectoplasm then is replaced by a cyst wall, glycogen accumulates in a central rounded mass (glycogen vacuole), and around this chromatoid bodies appear. The nucleus at first enlarges, then divides giving rise to a binucleate cyst. Through a second nuclear division the mature quadrinucleate cyst is formed. Trinucleate cysts, in which one nucleus has divided ahead of the other, are seen occasionally. The glycogen and chromatoid bodies are gradually consumed as the cyst matures and grows old. Trophozoites and cysts are carried away from the host with the fecal discharges in enormous numbers, up to 50,000,000 daily. The trophozoites are fragile animals and quickly die outside of the host unless kept moist and in the osmotic pressure of tissue fluids. Any dilution of the feces quickly kills them. They rarely succeed in reaching a new host and, if so, most likely succumb in the gastric secretion.

The cysts, on the other hand, are well designed for the purpose of transfer to new hosts, particularly via water in which they may survive for several weeks. The mature ingested cysts, after passing unharmed through the stomach and some distance down the small intestine, probably to the lower ileum, undergo excystation. Immature cysts probably do not excyst and are eliminated in the feces. The ameba within the cyst begins to move and presently escapes through a pore in the cyst wall. After a short period of feeding and growth, this metacystic ameba begins to divide. Each of the four nuclei divides once, and a part of the cytoplasm is split off for each nucleus, thus resulting in eight small amoebulae. These small amoebulae pass into the colon and, if conditions are favorable, grow and parasitize their new host.

Entamoeba coli

The life cycle of E. coli is similar to that of E. histolytica with these exceptions:

1. It does not invade the tissue of its host but lives in the lumen of the colon.

2. During encystment there are three instead of two successive nuclear divisions, giving eight instead of four nuclei in the mature cyst.

3. There are no nuclear divisions in the metacystic ameba; cytoplasmic fission occurs until one uninucleate amoebula is produced for each nucleus.

Endolimax nana

This parasite lives harmlessly in the lumen of the colon like E. coli. During encystment there are two successive nuclear divisions resulting in a quadrinucleate cyst as in E. histolytica. Encystation is not accompanied by the formation of large chromatoid bodies as seen in the endamoebae and only occasionally do glycogen masses appear.

Iodamoeba bütschlii

This is also a commensal living harmlessly in the lumen of the colon. The nucleus undergoes a slight change in its structure during encystment but does not divide. Both trophozoites and cysts are thus uninucleate. No large chromatoid bodies are formed, but the cytoplasm may be alveolar or granular. Glycogen is stored in a characteristic dense ball-like mass.

Dientamoeba fragilis

Like E. coli, E. nana, and I. bütschlii, this organism lives in the lumen of the colon without invading the tissues. It does not encyst, at least not in the human host. The method of transmission to a new host, without the resistant cyst stage, is not known. An animal reservoir, in which encystation takes place and from which humans are infected, is possible.

Giardia lamblia

This flagellate, unlike the amebas and the other flagellates, lives in the small intestine. It reaches as high as the duodenum and may be recovered by duodenal drainage. G. lamblia is provided with a sucking disc which appears to be an organ for attachment to the mucous membrane. It is actively motile, however, and presumably spends part of its time swimming about in the bowel lumen. It reproduces by longitudinal division and thereby maintains a colony. It encysts freely, probably in the distal part of the ileum. The various organelles become duplicated within the cyst, and two trophozoites are liberated with excystation. Transmission to new hosts is effected by the cysts through fecal contamination of food and water, as in the other cyst-forming species. Excystation takes place in the small intestine.

Chilomastix mesnili

This organism lives in the lumen of the colon and possibly in the distal part of the ileum. Like G. lamblia, it reproduces by longitudinal binary fission and encysts profusely. No nuclear division or other process of multiplication takes place within the cyst.

Trichomonas hominis

The habitat is in the colon. It reproduces by longitudinal fission, like the other flagellates, but it does not encyst in man. The trophozoites in a modified form may survive in the feces for a week or more and may be sufficiently resistant to reach new hosts via the various anus-to-mouth channels.

Retortamonas intestinalis and Enteromonas hominis

Little is known regarding the life cycle of these very small and rare flagellates. Both reproduce by binary fission and both encyst. The cyst of R. intestinalis is uninucleate, that of E. hominis is quadrinucleate.

Balantidium coli

This ciliate is an intestinal parasite of pig and man. In the former it is common and apparently harmless; in man it is very rare but sometimes pathogenic. It lives in the lumen of the colon feeding on fecal debris and, when available, blood and tissue cells. It may invade the bowel wall causing abscesses and dysenteric symptoms. It multiplies by transverse binary fission and is capable of a sexual process called conjugation. Encystment occurs very rarely in the human host. Man is infected by ingestion of cysts in the fecal discharges of man or pigs.

Isopora hominis and I. belli

This is the only genus of the Coccidia that has been definitely shown to parasitize man. Little attention has been given to differentiation of the two species. As with E. histolytica, size is the principal criterion for morphological differentiation. The life cycle is unknown but is believed to be similar to that of I. felis in the cat in which intracellular stages occur in the epithelial cells of the intestinal wall. The oocysts of I. hominis are passed in the feces. They are very resistant and may remain viable for a long time outside the body. They are transmitted to man by contaminated food or water. When ingested, the oocysts probably rupture and liberate sporozoites which penetrate the intestinal cells. Multiplication takes place within these cells and more oocysts are formed. Human infections are not common.

LABORATORY DIAGNOSIS

I. Collection of Specimens

Stool Specimens

The collection of fecal specimens is usually a nuisance to the patient and not a very pleasant task for nurses and others in attendance. It is, therefore, important to make this procedure of as little inconvenience as possible. In general, the normally passed stools are quite satisfactory. They are usually formed, mushy, or both, and therefore cyst-bearing stools rather than trophozoite-bearing. These stools are the best for routine work in a clinical laboratory. The use of cyst-bearing stools also has the practical advantage of eliminating, with few exceptions, the need for "fresh" and postcatharsis stools and all the inconvenience of collecting such specimens.

Fresh stools - Cyst-bearing stools need not be fresh since cysts remain practically unchanged for several hours. They may be examined at the convenience of the laboratory sometime during the day. If examination has to be postponed to the following day, the stool should be kept in the refrigerator to retard degenerative changes. The only indication for requesting a fresh stool is when the identification of E. histolytica has to be made on the trophozoite stage, e.g., in cases of dysentery when the patient cannot be expected to pass stools containing cysts. A fresh stool may then reveal trophozoites in active motility which together with other findings may permit a positive identification. The beginner may also need fresh stools for identification of D. fragilis and T. hominis which have no cysts.

Cathartics - Since normally passed stools are deemed the most desirable for routine work, there is obviously no place for catharsis except for special purposes:

1. It may become necessary when the object of the examination is not only a search for the principal pathogen, E. histolytica, but for all species. If during such a search, the patient passes only formed stools, a cathartic is indicated in order to obtain a mushy stool and with it the species which do not have cysts - D. fragilis and T. hominis. Formed cyst-bearing stools which are soft and moist may, like mushy stools, contain not only cysts but also trophozoites, usually few and degenerated, including D. fragilis and T. hominis.

2. Some workers have found that the intestinal protozoa, including the cyst-forming species, are more likely to be found in mushy rather than in formed stools. Keeping this fact in mind, one may resort to a cathartic when a patient passes only firm formed stools and when there is some special indication for a most thorough search.

In administering a cathartic for these purposes, it is important to remember that the object is to produce a mushy and not a watery stool. Cascara Sagrada in moderate dosage is one of the best. Castor oil or liquid petrolatum should not be used. These substances appear in the feces as tiny refractile globules, and although they float above the level of fecal debris and protozoa, they interfere with the search and confuse the beginner.

Chances of detecting the intestinal protozoa in feces - The number of trophozoites and/or cysts passed out with the feces varies greatly. One day there may be so many that a single smear under a 22 x 22-mm. cover slip may show more than a thousand, and a day or two later several smears may have to be searched before a single protozoan can be found. In some cases there may be intervals of several days when none can be found even if extended search is made. Whether numerous or scanty, the chances of detecting the intestinal protozoa in the feces depend mainly upon the skill and interest of the individual worker. The expert doing routine examinations will find more than 75% of the infections in a single specimen of feces, and with very few exceptions he will discover all infections in a series of three stools, at least one of which would have to be mushy in consistency. Laboratory workers who have not had adequate instruction discover only the most obvious forms, such as large E. coli cysts, the actively motile flagellates, or the giant trophozoites of E. histolytica crawling across the field in dysenteric discharge. Technicians who have not had their eyes opened to protozoan life may examine thousands of stools without ever seeing a cyst or trophozoite. No rule as to the number of stools that should be examined to determine the presence or absence of protozoan infection can therefore be made unless the qualifications of the worker are known.

Proctoscopic Specimens

Fecal material obtained by a proctoscope should be examined as soon as possible. It is often convenient for the technician to set up his microscope in the examining room and make his preliminary findings there. Slides used in the examination should be approximately body temperature. Use of cotton swabs should be avoided.

Blood Specimens for Complement Fixation

Draw 10 ml. of blood and place it in a sterile tube. Keep it under refrigeration. When clotted, break the clot, spin down, and remove the serum into a chemically clean tube. Pack and send the serum to a diagnostic laboratory for a complement fixation reaction.

II. Laboratory Methods Used in the Identification of Intestinal Protozoa

Many general technics are employed in detecting and identifying intestinal protozoa. They vary in applicability, depending upon the species of protozoan involved and the technical ability of the laboratory personnel. They should include a temporary mount, a permanent mount, and a flotation procedure. It is recommended that several of the first seven methods be employed routinely by all laboratories. The last three technics are useful only in specific and selected cases, and it is doubtful if they are of sufficient value, considering their technical refinements, to be used in any but diagnostic centers.

A. Fresh Specimens

1. Fresh Specimens in Isotonic Solution (normal saline with or without iron-hematoxylin).

Advantages: The easiest and best method for the detection of intestinal protozoa. Both trophozoites and cysts are usually alive and unaffected.

Disadvantages: The success of the method depends upon the skill of the examiner. Light infections are frequently missed. The preparation is not permanent.

2. Fresh Specimens in Hypotonic Solution (tap water)

Advantages: Objects which may be mistaken for protozoan cysts are eliminated. Some species behave characteristically in hypotonic solutions.

Disadvantages: Trophozoites are destroyed. Preparation is not permanent.

3. Fresh Specimens in a Staining Solution (iodine, eosin, etc.)

Advantage: Certain species are more easily identified when stained by iodine, etc.

Disadvantages: Protozoans are not so easily found. Trophozoites are lost. Preparation is not permanent. Technic does not aid in the identification of the majority of intestinal protozoa.

B. Fixed Specimens

4. Stained by Hematoxylin

Advantages: Most certain method for the identification of intestinal protozoa. Preparation is permanent.

Disadvantages: Time consuming. Protozoa are often difficult to find.

C. Combined Fixation, Preservation and Staining of Fecal Specimens

These technics combine the favorable features of other diagnostic procedures.

5. MIF Technic (M erthiolate F ormalin I odine

Advantages: Fixation and staining of fecal specimens which may be examined immediately or may be retained for permanent record and study. It is simple, reliable and permanent and there is no distortion of parasites.

Disadvantages: A few mature cysts in some preparations may be refractory and fail to take the stain immediately.

6. Rapid Permanent Mount Stain Technic

Advantages: Same as for the MIF technic, the entire procedure for preparation of permanent smear requires only 2 1/2 minutes.

D. Concentration of Specimens

7. By Flotation or Centrifugation

Advantage: Highest percentage of positives.

Disadvantages: Time consuming. Trophozoites lost. Small cysts very difficult to find. Preparation not permanent.

E. 8. Culture

Advantage: Picks up very light infections of some species.

Disadvantages: Time consuming. Only applicable to a few species.

F. 9. Complement Fixation

Advantages: Of immense value in cases of amebic hepatitis and in amebiasis with foci elsewhere than in the gastro-intestinal tract. Also useful in cases of chronic amebiasis.

Disadvantages: Antigens vary in potency. A considerable percentage of carriers are missed.

G. 10. Animal Inoculation

Advantages: Gives some idea of the virulence of the strain. Maintains strain for further study.

Disadvantages: Time consuming. Technic extremely difficult. Many protozoa cannot be established in laboratory animals.

III. Laboratory Procedure and Routine

Technics one, three, four, five, six and seven are employed routinely in the parasitology laboratory of the Naval Medical School. The last three methods are applied only in special cases. The MIF technic is highly recommended both for the routine laboratory and for use in field work of the survey type. It may be used for preservation of fresh stool specimens in the field, on the ward or in the home prior to transmittal to the laboratory. An accurate log must be kept of each specimen and all positive and negative findings recorded.

IV. Detection and Identification of Intestinal Protozoa in Direct Smears in Isotonic Solutions

Arrangement of Microscope and Light

Illumination - Use the light from a good microscope lamp, rather than daylight. Use a ground glass filter to give an even distribution of light and to eliminate any undesirable color. Blue frosted glass filters are desirable.

Microscope - Always use the 16-mm. or low-power objective to find the protozoa, never the high-dry or 4-mm. objective. Open the iris diaphragm to its widest position and use the concave side of the mirror. Focus on the field, and then gradually lower the condenser until a point is reached where there is maximum refractility and minimum glare. This point can be determined only by experimentation. It varies for every microscope and every individual. Rotate the fine adjustment knob up and down, and all refractile objects in the field should sparkle. Intestinal protozoa will stand out in this light as refractile, shining bodies that are colorless or have only the slightest greenish or bluish tint. The beginner will find it difficult to pick up intestinal protozoa, especially forms less than 10 microns in diameter, but it is essential to learn to search with the lower magnification in order to save time and examine more material. Also the important quality of refractility is lost with the higher powers.

The 4-mm. objective is usually required to bring out the morphological features necessary for differentiation. Some smears will have so many refractile, shining bodies resembling intestinal protozoa when viewed with the 16-mm. objective that it may be more convenient to search with the high-dry lens. The procedure avoids changing objectives constantly to identify suspicious-looking objects. No matter how devoid of intestinal protozoa or protozoan-like objects the smear may appear to be under the 16-mm. objective, the technician should always check his findings by searching a few times across the smear with the high-dry lens. At times he may then find that he has missed small trophozoites or cysts (8 microns or less).

Method of searching a smear - In order to cover a smear adequately and to avoid searching the same area more than once, the search should be made as if the smear were ruled and as indicated in the following diagram. Begin as one does in reading, at the left upper corner (actually the right lower corner of the slide since images in the microscope field are inverted and reversed). A mechanical stage is essential for this purpose. The importance of a careful search cannot be emphasized too strongly; undoubtedly, most mistakes in the examination for intestinal protozoa are due to failure to find rather than failure to identify.

Using the 16-mm. objective, a smear under a cover glass 22 x 22 mm. requires 11 round trips which comprise 200 or more fields. If the 4-mm. objective were used, four times as many round trips (44) would have to be made, involving more than 3,000 fields.

This procedure is followed in examining all wet preparations.

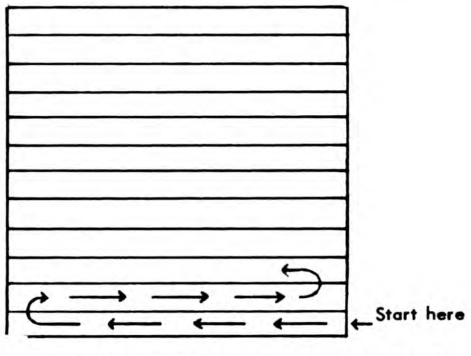


Fig. 8 - Diagram illustrating method of searching a slide

Solutions

1. Normal physiological saline.

2. Saline-Iron-Hematoxylin (Lawless).*

Add 15 ml. of 0.5% hematoxylin stain solution and 0.25 ml. (about 10 drops) of 4% ferric ammonium sulfate (see pages 52-53) to 75 ml. of normal saline. Shake each morning before using.

Technic

Place a drop of salt solution on a slide. Select a small particle of feces with a toothpick or applicator and stir it in the drop of salt solution until it forms a smooth emulsion. Apply a cover slip, and in so doing avoid air bubbles by placing the cover slip perpendicularly in the emulsion and moving it along the slide for a distance of about an inch, carrying the preparation with it. This wets the surface of the slide to be covered. Then move the cover slip, still held perpendicularly, back to the original position and lower it gently on the emulsion. The film should be thin and uniform so that when seen under the microscope the various fecal particles and the protozoa will be separated and clearly illuminated. In thicker films the protozoa are frequently missed, and if found, they are partially hidden in the fecal matter and difficult or impossible to identify. The importance of using thin smears applies particularly to the beginner who invariably will find more protozoa in the thin films although they contain less fecal material and fewer animals. A too large drop of saline will also give an unsatisfactory preparation. This can be corrected by touching the edge of the film with the corner of a piece of filter paper to absorb the excess fluid. An emulsion, when properly prepared, will allow ordinary newspaper print to be barely legible through the preparation.

The saline solution sometimes becomes contaminated with free-living protozoa which may be mistaken for intestinal forms. To guard against this, use a fresh solution or make a smear of the solution occasionally and examine for contamination.

Purpose of the Direct Smear in Isotonic Solution

The wet smear in physiological salt solution is intended for the study of protozoa in the living condition. Not all the morphological features on which the identification is made can be seen in the living protozoa. In spite of this limitation, the normal saline smear is most valuable in the examination of feces for intestinal forms. It is routinely the first preparation to be made and examined. It is the preparation used to determine whether a specimen is negative, positive, or probably positive, and it is, with few exceptions, the best preparation for this purpose. If the normal saline smears are negative, revealing no suspicious-looking objects which might be protozoa, no other preparation need be examined. At least two smears should be examined from a "routine" stool specimen, one from the outside and the other from the inside of the stool mass. In clinical cases with a history or findings

*Ref. Lawless, D.K.: Detecting Intestinal Protozoa; Saline-Iron Haematoxylin Solution for Wet Smears; Am. Jour. Trop. Med., 1946; 26:133-134

pointing to gastro-intestinal involvement, several additional preparations should be examined.

The saline-iron-hematoxylin smear is a modification which has been developed at the Naval Medical School. It has definite advantages, especially for the worker inexperienced in detecting intestinal protozoa. The solution tends to clump or coagulate the fecal debris and produces a stained background. The cysts and trophozoites remain free of debris in a clear field. Their refractility actually, or due to contrast, is increased. The activity of the trophozoites is unchanged.

Ameba Trophozoites in Direct Smears in Isotonic Solutions

General appearance - Under low magnification (16-mm. objective), the living unstained trophozoites appear as highly refractile, shining bodies from the size of the smallest pinhead to that of a buckshot. With the 4-mm. objective, the clear, homogeneous ectoplasm with its pseudopodia, the granular endoplasm with some of its inclusions, and sometimes a nucleus can be seen. Higher magnification with an oil immersion lens does not reveal additional features of value.

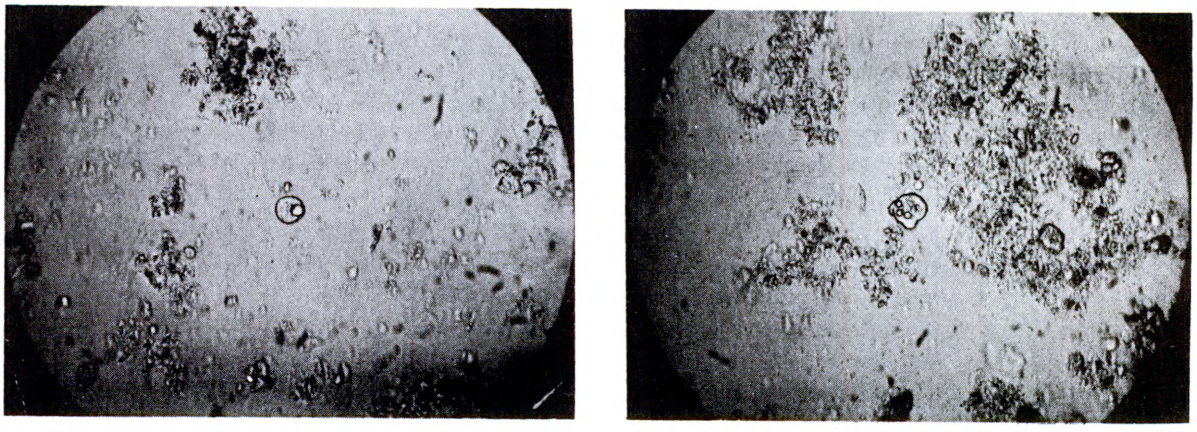


Fig. 9 -- E. coli trophozoites containing starch granules

Size - As indicated in the table (page 24), the trophozoites of each species have a rather extensive size range with considerable overlap. The distribution, however, within the size ranges is not uniform. The great majority in any one specimen vary only 2 or 3 microns. The eye has difficulty in appreciating a difference of 2 microns or less (except in small forms under high magnification); the usual impression is, therefore, that the trophozoites are about the same size,

except for some dwarfs and giants. This uniformity in size of the majority is of practical importance in differentiation; it is possible to group the trophozoites as follows:

Small, less than 10 microns
- E. histolytica (small race)
- E. nana

Medium to small, 10 to 12 microns
- I. bütschlii
- D. fragilis

Medium, 12 to 14 microns
- E. histolytica (from nondysenteric cases)

Large, 18 or more microns
- E. histolytica (large race from cases of amebic dysentery)
- E. coli

D. fragilis, when growing luxuriantly, sometimes defies the rule of size uniformity of the majority and may then range in almost uniform distribution from 6 to 16 microns.

Shape - Motile trophozoites obviously vary in shape as pseudopodia are protruded and retracted. They have a rounded but slightly irregular contour when at rest, except D. fragilis which is distinctively spherical with a faultlessly circular outline, as if drawn by a compass.

Motility - The form of motility most commonly seen consists of apparently purposeless extensions (pseudopodia) and retractions of the ectoplasm in various directions. These pseudopodia may be tongue-like, crescent-shaped, budding, or knob-like and are not always characteristic of the species. There are, however, two forms of pseudopodial activity which are of distinctive and practical value in the identification of species: (1) The finger-like, explosive pseudopodium, giving a progressive directional crawl to E. histolytica (imitated by E. coli on very rare occasions) and (2) the thin, veil-like pseudopodia of D. fragilis with their sharp corners or points (very rarely observed).

In the type of motility described as "progressive directional crawl," there is a rapid succession of ectoplasmic projections mainly in one direction. The animal becomes elongated, ribbon-like, with an anterior advancing and a posterior dragging end to which a brush of debris is frequently attached. As the ectoplasm advances, the endoplasm flows forward. Sometimes these motions become so lively that the action appears continuous. When this typical motility is seen in small trophozoites (10 microns or less), it identifies them as E. histolytica; in medium-sized and large trophozoites, it also identifies E. histolytica with very rare exceptions. If subsequent stools disclose E. coli cysts, one would have to admit that E. coli had assumed unusual motility.

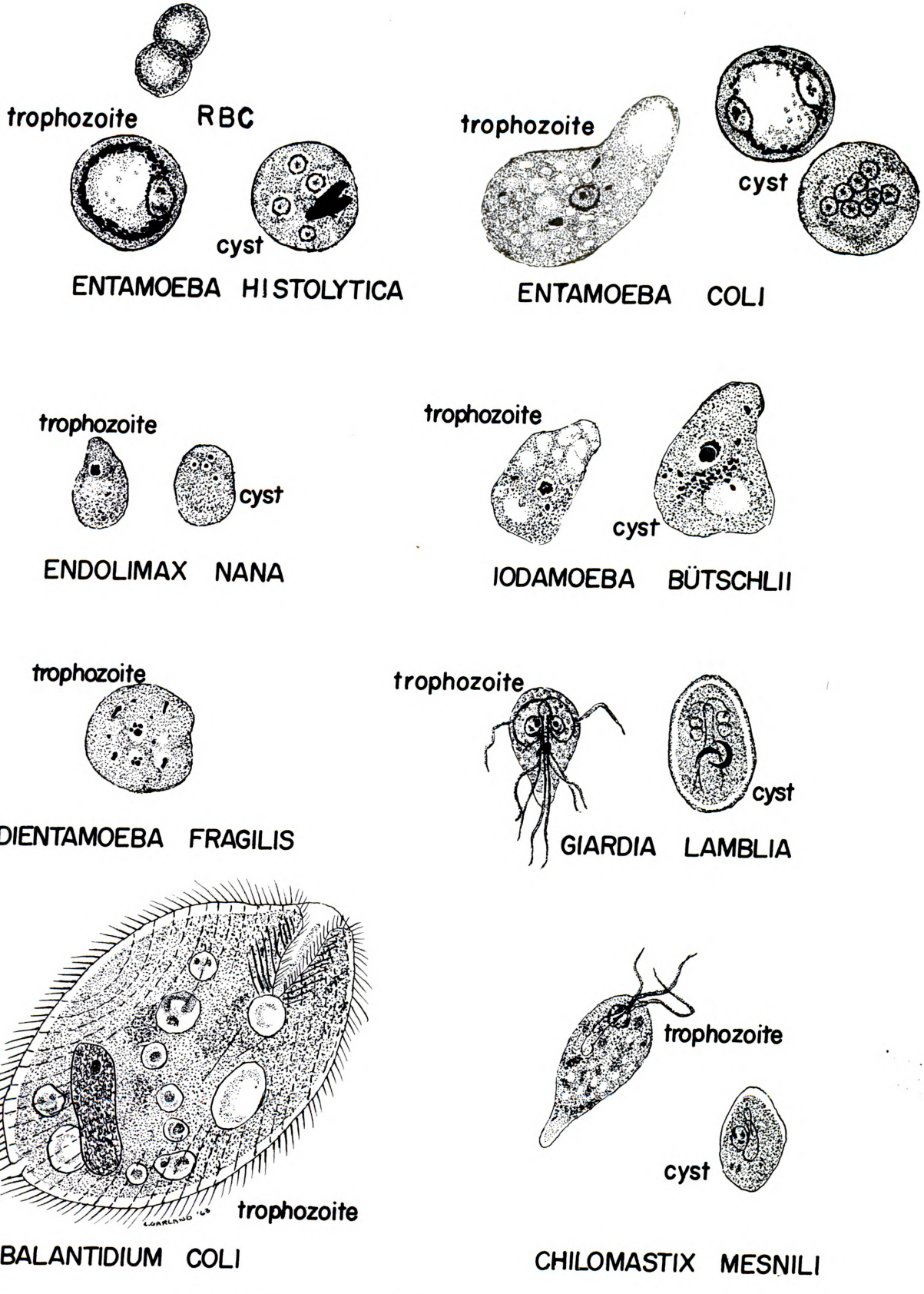


FIG.10 **INTESTINAL PROTOZOA OF MAN**

Cytoplasm and its inclusions - The cytoplasm of E. coli usually contains numerous ingested bacteria which are absent in E. histolytica. Red blood cells are rarely, if ever, ingested by E. coli; when these are found in the trophozoite, they are diagnostic of E. histolytica. There are some minor differences, such as the uniformity of the endoplasm in E. histolytica and its thinness in D. fragilis, but there are many exceptions to these.

E. histolytica with ingested erythrocytes is seen in the stools of patients with acute or subacute amebic colitis. The ingested red cells are usually in various stages of digestion and absorption, fading in color and shrinking in size. The beginner sometimes mistakes yeasts, starch granules, and other food inclusions, and even vacuoles of degeneration for ingested erythrocytes. The important distinguishing feature is the salmon-pink color of the erythrocyte. Unfortunately a trophozoite with ingested red cells is not always E. histolytica; it may be an E. coli. The ingestion of erythrocytes by E. coli is, however, a very rare occurrence. It has been observed only a few times and then in patients with persistent bleeding into the colon (bleeding papilloma in one case) during which E. coli apparently became conditioned to feeding on red cells.

To eliminate the possibility that trophozoites with ingested red blood cells may be E. coli, there are two procedures which are quite convenient for a clinical laboratory:

1. Look for cysts. They are easily identified, whether E. histolytica or E. coli, and unless the stool is quite liquid or merely an exudate, they will be present.

2. Look for trophozoites in progressive crawling motion (a fresh stool may be necessary for this purpose). When trophozoites crawl through the field and when some or all contain erythrocytes, one may without hesitancy identify them as E. histolytica. (The chance that an E. coli would present at the same time two features, both extremely rare for this species, would be too slim to be of practical significance.)

Visibility of nuclei - The nuclei are visible in E. coli trophozoites, but in E. histolytica only on rare occasions when the nucleus happens to be in the extended thin pseudopodium. The visibility depends upon the state of health of the trophozoite. In degenerated forms, the fine chromatin granules on the nuclear membrane have coalesced into irregular masses, large enough to be visible even in E. histolytica. Trophozoites damaged to this extent are sometimes present in fresh stools.

Procedure when trophozoites cannot be identified - The first step, and frequently the only one necessary, is to search for cysts. The cysts, as will be shown below, are relatively easy to identify. Some cysts can usually be found in the specimen that showed the trophozoites, unless liquid in consistency. If no cysts are found, there are two ways of proceeding:

1. Request additional specimens until a cyst-bearing stool is obtained. (Unless the patient has an acute dysentery or is passing liquid stools from purgation or food poisoning, there is usually no difficulty in obtaining a cyst-bearing stool.)

2. Make stained smears. These will readily differentiate the two Entamoebas from the other amebas, but, unless faultlessly stained and examined by an experienced worker, they may not make differentiation between E. coli and E. histolytica possible.

In the absence of ingested red blood cells, it may be impossible to differentiate between the trophozoites of E. histolytica and E. coli. In such a case, it may be indicated to render the patient constipated in order to obtain cysts. Trophozoites are usually found only in liquid or soft stools, while cysts may occur in stools of any consistency.

Key to the Trophozoites of the Intestinal Amebas in Isotonic Smears

1. Trophozoites large, more than 18 microns. 2
   Trophozoites small, less than 18 microns 3

2. Trophozoites with progressive directional crawl or with ingested red blood cells*; cytoplasm clean, without ingested bacteria; nucleus rarely visible. . . . . Entamoeba histolytica
   Trophozoite with sluggish movement; cytoplasm dirty, with ingested bacteria and other food material but not with ingested red blood cells*; nucleus always visible . . . . Entamoeba coli

3. Trophozoites motile. 4
   Trophozoites nonmotile 5

4. Trophozoite with progressive directional crawl . . . . . Entamoeba histolytica
   Trophozoite with angular, thin, veil-like pseudopodia . . .Dientamoeba fragilis (confirm in tap water smear)

5. Trophozoites all faultlessly spherical, resembling cysts, with ingested food and bacteria . . . . . .Dientamoeba fragilis (confirm in tap water smear)
   Trophozoites irregular in shape . . . . . species undetermined

All tentative determinations must be confirmed in a stained (haematoxylin or preparation by MIF or by Rapid Permanent Mount Stain Technic) smear.

Ameba Cysts in Direct Smears in Isotonic Solutions

General appearance - The living cysts, like the trophozoites, appear as refractile, shining objects under low magnification (16-mm. objective). With the

*See text for the rare exceptions to this statement

DIFFERENTIAL CHARACTERISTICS OF THE INTESTINAL AMEBAS
LIVING TROPHOZOITES
(normal saline smear)

	E. histolytica	E. coli	E. nana	I. bütschlii	D. fragilis
Size (in microns)	6 to 40; small race: av. 8; large race: in "carriers" av. 14; in dysentery av. 24	12 to 30; usually about 20	6 to 12; usually about 8	6 to 20; usually about 10	6 to 18; usually about 12
Shape when at rest	Rounded but slightly irregular	Same	Same	Same	Faultlessly spherical (distinctive)
Motility	Pseudopodia tongue-like, protruded with explosive force, sometimes in one direction and in rapid succession, giving rise to a progressive directional crawl (force and crawl diagnostic); in cold forms, pseudopodia similar to E. coli and E. nana	Pseudopodia broad, crescent-shaped, sometimes budding, sluggishly protruded, and lazily withdrawn; rarely in sluggish, progressive directional crawl	Pseudopodia small, budding, knob-like, overlapping; little or no progression	Same as E. coli, never in progressive directional crawl	Thin, veil-like, fan-shaped, triangular, or rectangular, or leaf-like with sharp corners or points (diagnostic); no progression
Endoplasm and its inclusions	Endoplasm "clean"; R.B.C.'s ingested in the presence of blood, as e.g. in amebic dysentery (diagnostic)	Endoplasm "dirty" due to bacteria and food debris; ingested R.B.C.'s seen very rarely	Same as E. coli; no ingestion of R.B.C.'s	Same as E. coli; no ingestion of R.B.C.'s	Cytoplasm thin; ingested bacteria and food show clearly; no ingestion of R.B.C.'s
Visibility of nuclei	Invisible except in flowing endoplasm during progressive crawl when it may be seen as a dimly outlined ring; in old trophozoites, degenerated nucleus clearly visible as a black ring	Almost always visible as a grayish or black ring, even in "fresh" trophozoites	Sometimes seen as a grayish-blue disc; (not clear enough to be of diagnostic importance)	Same as E. nana	Not visible

4-mm. objective, they can usually be distinguished from the trophozoites on their less granular, sometimes quite uniform cytoplasm and the cyst wall which in optical section has the appearance of a smooth steel wire.

Size - The cysts show less variation in size than the trophozoites, and the uniformity in size of the majority is more obvious. They are grouped by the eye as small, medium-sized, large, and variable:

Small, less than 10 microns
- E. histolytica, small race
- E. nana

Medium-sized, about 12 microns
- E. histolytica, large race
- E. coli (very rare)

Large, about 18 microns
- E. coli

Variable, 6 to 16 microns
- I. bütschlii (usually around 10 microns)

Shape - E. histolytica and E. coli cysts tend to be perfectly round although forms of unusual shape may be seen, especially in the latter. I. bütschlii forms are only rarely round, the odd-shaped cyst predominating. A small ovoid- or sausage-shaped cyst is almost always E. nana. If seen on end, these cysts appear round, and they may be impossible to differentiate from small race E. histolytica.

Number of nuclei - The nuclei can often be seen in an E. coli cyst. It may not be possible to distinguish all of them, but this is not necessary since a count of five or more will differentiate the cyst from E. histolytica. (Supernucleated E. histolytica cysts with five to eight nuclei occur, but too infrequently to be of any practical importance.)

Cytoplasmic inclusions - Both large and small race E. histolytica are practically identified by the chromatoid bodies. In the large race, they appear as refractile glassy rods or bars. The small chromatoid granules in immature cysts which show in stained smears are not visible. The characteristic rods are usually seen in about one-half of the cysts. In the small race cysts, the chromatoid bodies are very tiny, but they are brilliantly refractile, and the experienced worker has no difficulty seeing them under the 4-mm. objective. They are usually present in well over 50% of the cysts. Should no chromatoid bodies be seen, small race

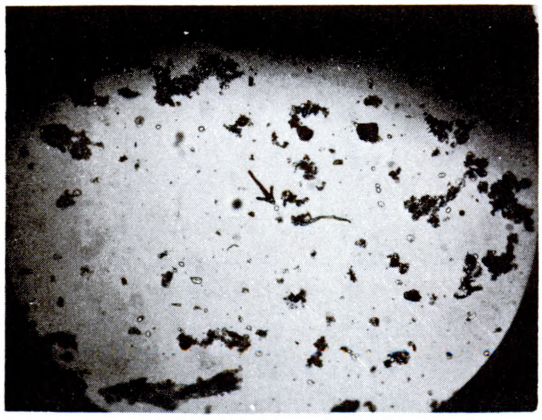


Fig. 11

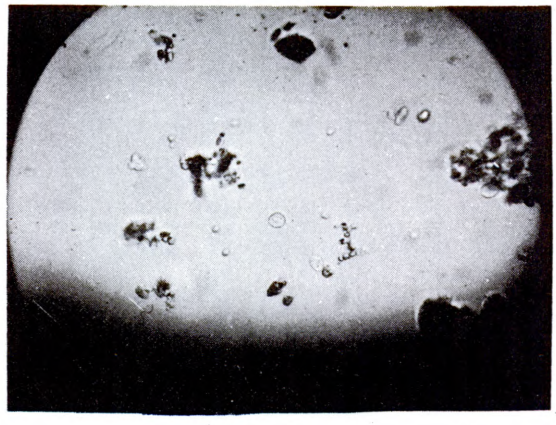


Fig. 12

E. histolytica cysts, 16- and 4-mm. objectives

E. histolytica cysts are usually mistaken for E. nana. To guard against this error, it is well to suspect any batch of "small" (10 microns or less) cysts of being small race E. histolytica. Occasionally the chromatoid bodies of E. coli cysts, although so distinctively splintered when stained, may appear rod-like to the inexperienced. As a rule, however, careful focussing reveals stranding or needle points. As an added safeguard, make an iodine smear to reveal the nuclei. Their number and arrangement permit an immediate differentiation.

The ball-like glycogen mass and clusters of volutin granules identify I. bütschlii. No other cysts in human feces have their glycogen similarly concentrated in a dense, sharply limited mass.

DIFFERENTIAL CHARACTERISTICS OF THE INTESTINAL AMEBAS
LIVING CYSTS
(normal saline or aqueous smears)

	E. histolytica	E. coli	E. nana	I. bütschlii	D. fragilis
Size (in microns)	5 to 16; small race about 8; large race about 12	10 to 30; average 17	6 to 10; usually 8	6 to 16; usually 10	No cysts
Shape	Rounded but seldom perfectly spherical	Same	Rounded, oval, or sausage-shaped (diagnostic)	Rounded or odd-shaped; triangular, pear, or kidney-shaped (diagnostic)	No cysts
Chromatoid matter	Abundant; bars with rounded ends or cigar-shaped rods (diagnostic); present in from 0 to 100%, usually in more than 50% of the cysts	Scanty; spicules or blocks with sharp points (diagnostic); seldom seen in more than 10% of the cysts	None	None	No cysts
Glycogen	Rarely in sufficient amount to change appearance of cyst	Young cysts filled with glycogen have muddy cytoplasm	No glycogen visible	Majority have well-defined dense glycogen ball, slightly refractile, light blue (diagnostic)	No cysts
Volutin granules	None	None	None	Clusters of black granules usually present (diagnostic)	No cysts
Visibility of nucleus	Not visible except in old degenerated cysts	Visible except when concealed by glycogen in young cysts; more than four is diagnostic	Not visible	Sometimes visible as a light-blue disc	No cysts

Key to the Cysts of the Intestinal Amebas in Direct Isotonic Smears*

1. With ball-like refractile glycogen mass and odd or distorted shape. . . . . Iodamoeba bütschlii**
   Without glycogen ball; usually faultlessly spherical or oval 2

2. Large, 18μ or more in diameter. . . . . Entamoeba coli
   Medium-sized, from 10μ to 17μ in diameter 3
   Small, less than 10μ in diameter 4

3. With rod-shaped, chromatoid bars with rounded ends . . Entamoeba histolytica.
   With chromatoids with splintered ends or with more than four nuclei. . . . . Entamoeba coli**
   Without chromatoid bodies and one to four nuclei. . . . . Entamoeba species

4. Oval or sausage-shaped, without chromatoid bars . . . . . Endolimax nana
   Round, with chromatoid bodies . . . . . Entamoeba histolytica

Caution - Although it has no cysts, Dientamoeba fragilis may be mentioned here because the beginner, seeing the immotile forms with their circular outlines, frequently mistakes them for cysts, usually E. histolytica.

*Confirm all identifications in smears, stained with haematoxylin, by MIF or by the rapid stain technic.
**Confirm in iodine smear.

Flagellates and Ciliate Trophozoites in Direct Smears in Isotonic Solutions

Giardia lamblia, living trophozoites - The trophozoites of G. lamblia are rarely seen in the feces. They have their habitat in the small intestine; those which are carried along with the intestinal contents through the colon and out with the feces have usually either encysted or died and disintegrated. To bring them down, the rapid flow of intestinal contents incident to diarrhea or purgation is usually necessary. Even then most of them are dead or dying. Whether dead or alive, if once seen, they can always be easily recognized. Their motility, a slow, tumbling, or falling-leaf movement, is distinctive; when immotile, the "skeleton" (the nuclear rings, the axostyle, and the tail which are visible even when unstained) invariably reveals their identity.

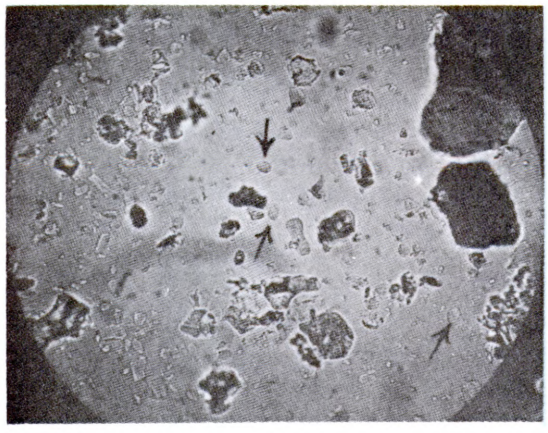


Fig. 13 - Size relationship of G. lamblia as observed in the microscope field. (magn. approx. 200 X)

Chilomastix mesnili, living trophozoites - This flagellate is cone-shaped and has a spiral groove encircling the body, giving it the appearance of a twisted cone. It seems to traverse the field like a torpedo under the 16-mm. objective. With the 4-mm. objective, it can be seen boring ahead with slow rotation of the body, displaying in cycles the deep cleft of the cytostomal groove. Small, rounded, abnormal forms (5-7 microns) of C. mesnili may be present in the stool, but careful search will always disclose one or more normal Chilomastix.

Trichomonas hominis, living trophozoites - The ovoid shape and the manner of motility - rapidly repeated, quick jerks - suffice for recognition by the experienced observer. The student should also note the undulating membrane and the lashing flagella before making a positive identification. These organelles can best be seen in sluggishly moving individuals. A faint trace of iodine added to the normal saline smear may be necessary to retard their movements.

As T. hominis grows old in the feces, it loses its flagella and undulating membrane and changes into a bag-like structure of homogeneous, elastic material. Along one edge of this bag there is an undulating movement which at times is interrupted by finger-like protrusions. The latter may be mistaken for pseudopodial movements and lead the observer to identify these forms as small amebas.

Retortamonas intestinalis and Enteromonas hominis, living trophozoites - These closely resemble T. hominis. Their smaller size and the absence of an undulating membrane are the important differential features.

Balantidium coli, living trophozoites - This large animal darting across the field can be recognized under the 16-mm. objective. Under the 4-mm. lens, the distinctive organelles can be seen: the hair-like cilia, the mouth (cytostome), vacuoles, and nuclei.

Flagellates, Ciliate, and Sporozoan Cysts in Direct Smears in Isotonic Solutions

Giardia lamblia, living cysts - These cysts are the only medium-sized, football-shaped cysts, except for an occasional out-of-shape entamoeba cyst (E. histolytica large race, E. coli small race). They also have another distinctive feature, namely, a narrow, clear space between the cyst wall and the encysted animal. In addition, nuclei, fibrils, and rods are visible in the cytoplasm, except in young cysts when these markings are obscured by glycogen deposits. When standing on end and then presenting a rounded outline, they are sometimes mistaken for E. histolytica cysts by the beginner who fails to note the clear space inside the cyst wall.

Chilomastix mesnili, living cysts - The cysts of C. mesnili are small, less than 10 microns, and thus within the size range of E. nana and small race E. histolytica. However, they need not be mistaken for these cysts since their shape is distinctive. No other cysts in the feces have the shape of a nippled lemon. It must be kept in mind that some cysts will be standing on end and hiding the nipple, presenting a rounded outline in optical section.

DIFFERENTIAL CHARACTERISTICS OF THE THREE COMMON INTESTINAL FLAGELLATES AND THE CILIATE

LIVING TROPHOZOITES
(normal saline smears)

	T. hominis	C. mesnili	G. lamblia	B. coli
Shape and size (in microns)	Ovoid; usually less than 10	Cone-shaped; usually 12 to 15 long; small form 5 to 8	Pear-shaped in antero-posterior view; spoon-shaped in lateral view; usually 12 to 15 long	Roughly oval; a large animal about 50 x 70
Motility	Appears nervous; moves with rapidly repeated quick jerks	Boring progressive motion with slow rotation of the body	Slow tumbling or falling-leaf motion in various directions but without much progression	Darts across the field; body in slow rotary movement
Structures seen under 4-mm. objective	Lashing flagella and undulating membrane (diagnostic)	Spiral groove (diagnostic)	Nuclei, parallel axostyles, and tail	Waving rows of cilia, cytostome, nuclei, and contractile vacuoles

LIVING CYSTS
(normal saline or aqueous smears)

	T. hominis	C. mesnili	G. lamblia	B. coli
Shape and size (in microns)	No cysts	Lemon-shaped with nipple-like thickening of wall at narrower end; about 8	Football-shaped (round when standing on end); about 8 x 12	Rounded; 50 to 60; rarely seen
Structures seen under 4-mm. objective	No cysts	Cytoplasm dense; no trace of nucleus or flagella seen	Narrow clear space between cyst wall and encysted animal; fibrils, rods, and rings in various designs except in young cysts, where they are obscured by glycogen	Thick double wall; conspicuous kidney-shaped macronucleus

Trichomonas hominis - No cysts.

Retortamonas intestinalis and Enteromonas hominis, living cysts - These are the smallest protozoan cysts, 6 microns or less. The shapes are characteristic: pear-shaped for R. intestinalis and barrel-shaped for E. homonis.

Balantidium coli, living cysts - These are very rarely seen in the human feces and then only in formed stools. Their large size (about three times as large as E. coli cysts), the thick wall, and the macronucleus are conspicious features which are readily seen.

Isospora hominis and I. belli - Only the oöcyst stage as seen in the feces is known. It is an ovoid structure, about 10 by 16 microns for I. hominis and 15 by 30 microns in I. belli, consisting of a glass-like wall enclosing a rounded granular mass of cytoplasm. The glass-like wall is practically invisible under the 16-mm. objective and appears only as a clear zone surrounding the granular masses of cytoplasm.

Positive Identifications Possible in Direct Smears in Isotonic Solutions

E. histolytica trophozoites - with ingested red blood cells or directional crawl.

E. histolytica cysts - containing rod-shaped chromatoid bodies with rounded ends.

E. coli cysts - with more than four nuclei or larger than 18 microns.

I. bütschlii cysts - with glycogen ball and odd shape.

D. fragilis trophozoites - with characteristic motility.

G. lamblia trophozoites - with characteristic pear shape and tumbling leaf motility.

G. lamblia cysts - with football shape, double wall, and visible inclusions.

C. mesnili trophozoites - with characteristic boring motion and spiral groove.

C. mesnili cysts - with characteristic lemon shape.

T. hominis trophozoites - with jerky motion and undulating membrane.

B. coli trophozoites - with characteristic cilia and large size.

B. coli cysts - large size.

V. Detection and Identification of Intestinal Protozoa in Direct Smears in Hypotonic Solution

Use of Microscope

Use as for smears in isotonic solutions.

Purpose of Method

The purposes of this method are twofold:

1. To eliminate all pseudoparasites which resemble cysts in the preparation. Protozoan cysts remain unchanged, but blastocysts and other confusing objects are destroyed. Trophozoites are also eliminated by this method and can thus be differentiated from cysts.

2. The distinctive manner in which D. fragilis ruptures is easy to recognize and frequently constitutes a practical means of identification. By the time the smear has been placed under the microscope, the dientamoebae have begun to distend. The endoplasm becomes thin, lusterless, and transparent. Bacteria and other inclusions become clearly outlined; the nucleus or nuclei can be seen with good illumination as dull refractile discs. The distention terminates in 1 to 10 minutes with an explosive rupture of the ectoplasm and expulsion of its contents. The ectoplasm then, like a rubber ball, quickly regains its spherical form. Some particles, and occasionally a nucleus, remain within this shell. The evacuated endoplasm quickly disappears, but the ectoplasm remains intact for several minutes. All three phases - the distention, the rupture, and the remaining ectoplasmic shell - must be observed in order to make a positive identification.

Solutions

Distilled or tap water.

Technic

A thin smear is made in tap water or distilled water in the same manner as a normal saline smear.

VI. Detection and Identification of Intestinal Protozoa in Direct Smears Stained by Iodine

Use of Microscope

Protozoa are much more difficult to find in iodine solution because they lose most of their refractility. The condenser, therefore, should be raised to its normal position just beneath the stage and the light controlled by the iris diaphragm.

Purpose of Method

In staining the nuclei, the iodine frequently fails to bring out the fine details on which species are differentiated. It merely shows the number of nuclei and their arrangement. It is of practical value only as noted below:

1. E. histolytica cysts, large race - In these the iodine will visualize quite clearly the number and arrangement of the nuclei and their ring-like character. In the small race of E. histolytica the iodine usually fails to show the delicate nuclear rings with sufficient clarity to permit identification.

2. E. coli cysts - The nuclei of these cysts usually show very clearly in the iodine smear and identification is practically always possible.

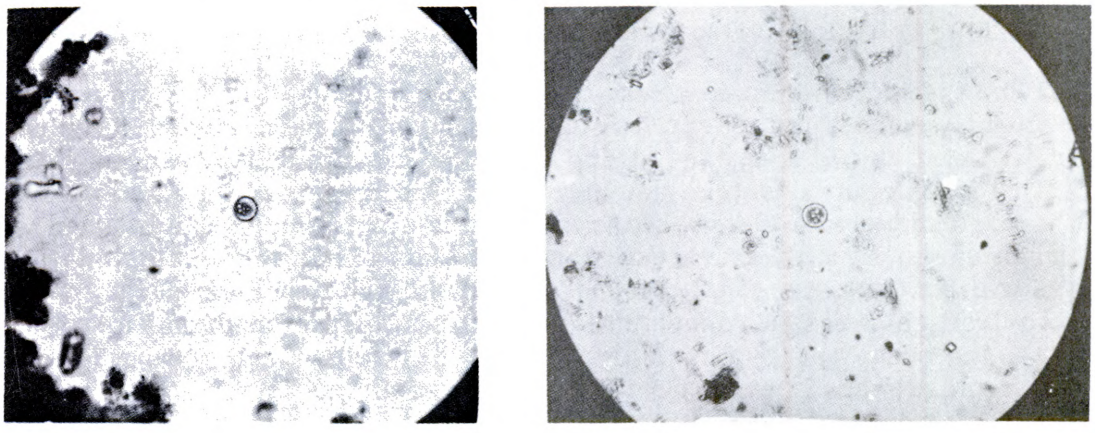


Fig. 14 - E. coli cysts, 4-mm. objective, iodine smears (Magn. approx. 200 x)

3. I. bütschlii cysts - The distinctive glycogen ball is sharply outlined in the iodine smear.

Fig. 15 - I. bütschlii cysts, 4-mm. objective, iodine smears

For the identification of the cysts of E. nana, C. mesnili, Retortamonas intestinalis, and E. hominis, the iodine smear is of no assistance, and the remaining cysts, G. lamblia and B. coli, can be recognized in the normal saline smear.

Solutions

Lugol's solution:

Iodine crystals	5.0 gm.
Potassium iodide	10.0 gm.
Distilled water, q.s.	100.0 ml.

D'Antoni's iodine solution: Prepare a 10% potassium iodide solution by the specific gravity method. From this reagent, a 1% solution is prepared, to which 1.5 gm. of iodine crystals are added for each 100 ml.

Technic

Make a smear as with saline, but use water in order to eliminate the blastocysts which would otherwise take the stain and be confused with protozoan cysts. Trophozoites will also be destroyed, but iodine staining is not used to identify them. Dip a toothpick or applicator in Lugol's solution and stir the smear until it is lightly stained, or add a drop of D'Antoni's iodine solution. An alternate technic is to tease the iodine solution under the cover slip after the preparation has been made and the protozoans found.

The iodine kills the trophozoites and the cysts. It stains the glycogen and nuclear chromatin first and then tints the cytoplasm, unless used in a very weak concentration. The object of the iodine smear is to stain the nuclei; therefore the critical point in staining is reached when the sharpest contrast between the nuclei and the surrounding cytoplasm and glycogen has been obtained. This usually requires the smallest amount of iodine that will stain nuclei. Increasing the amount of iodine will not only deeply stain the nuclei but will also heavily stain the cytoplasm and decrease the contrast. This applies especially to young cysts of E. histolytica and E. coli which usually contain much glycogen. With a minimum of iodine, the fecal material remains quite uniformly spread, and the lightly stained cysts retain some of their refractility so they may be picked up with the 16-mm. objective. In heavily stained smears, on the other hand, the fecal material is clumped by coagulation of mucus, and the cysts, having lost their luster, can rarely be found except after a tedious search with the 4-mm. objective.

VII. Detection and Identification of Intestinal Protozoa in Fixed Smears Stained by Iron-Hematoxylin

In order to visualize the appearance and structure of the various forms of intestinal protozoa, they must be seen both as living and as dead and stained. As

studied in the wet preparation, they reveal their size, shape, motility, and a few other characteristics. Frequently these suffice for identification. When fixed and stained with iron-hematoxylin, finer details of structure are revealed and positive identification is then almost always possible. The technic of this staining is given on page 50.

Use of Microscope

For searching a stained smear, the 16-mm. objective cannot be used except for selecting a promising field. Even with the 4-mm. objective the smaller forms are easily overlooked. For identification, the oil-immersion objective is necessary. Many workers use the oil immersion objective exclusively.

The student at first has difficulty in visualizing the protozoan cysts as spherical bodies, although they are seen rolling across the field in the wet preparations. The optical section produced by the microscope reveals only a horizontal plane through the cyst. The entire structure is seen only by changing the focus. The accompanying figure diagrams a cyst of E. histolytica as though it were transparent. Note that the nuclei as well as the cyst are presented in spherical shape. The chromatoid material is in round bars. The chromatin on the nuclear membrane is dispersed over the surface and is not limited to the perimeter of a circle.

Fig. 16 - Diagram of an E. histolytica cyst in three dimensions (magn. approx. 62,000 x).

IRON-HEMATOXYLIN STAINED PROTOZOA

Species	Diagnostic Features
Entamoeba histolytica	The histolytica nucleus; trophozoites with ingested red cells, "clean" cytoplasm; cysts with one to four nuclei, one and four predominant; chromatoid bars with rounded ends
Entamoeba coli	The coli nucleus; trophozoites with "dirty" vacuolated cytoplasm; cysts with one to eight nuclei, two and eight most common; chromatoid splinters with sharp ends occasionally seen
Endolimax nana	The Endolimax nucleus; trophozoites uninucleate, may resemble Iodamoeba bütschlii except for size; cysts with two to four dot-like nuclei
Iodamoeba bütschlii	The Iodamoeba nucleus; trophozoites may resemble E. nana except for size; cysts with the vacuole of the glycogen ball
Dientamoeba fragilis	The Dientamoeba nucleus; only trophozoites present; one or two nuclei
Giardia lamblia	Trophozoites binucleate, cysts quadrinucleate; visible flagella, axostyle, blepharoplast, and parabasal body
Chilomastix mesnili	Trophozoite with flagella and spiral groove; cysts uninucleate with visible fibrils in shape of safety pin
Balantidium coli	The Balantidium nucleus; trophozoite with visible cilia, cytostome; cyst with double wall

Nuclear Characteristics of the Intestinal Amebas

The appearance of the nuclei of the amebas in stained smears is of special importance. The differentiation of the four genera to which the amebas belong, Entamoeba, Endolimax, Iodamoeba, and Dientamoeba, is done mainly on the morphology of the nuclei, and, since there is only a single species of Endolimax, Iodamoeba, and Dientamoeba represented in man, the identification of these genera also identifies the species.

The Entamoeba nucleus has a relatively small karyosome and a layer of chromatin granules on the inner surface of the nuclear membrane. In optical section it resembles a cart wheel, the karyosome forming the hub and the nuclear membrane with its chromatin layer the rim. Sometimes lines of fine granules radiate from the karyosome to the periphery as spokes of the wheel. The nuclei of the two entamoebae, E. histolytica and E. coli, are quite similar. It is only when they are faultlessly stained before degenerative changes have occurred that they can be differentiated with certainty. The most important and most constant differential points are the fine uniform chromatin granules on the nuclear membrane and the small centrally placed karyosome in the nucleus of E. histolytica as compared with the large irregularly shaped chromatin granules on the nuclear membrane and the large eccentrically located karyosome in the nucleus of E. coli.

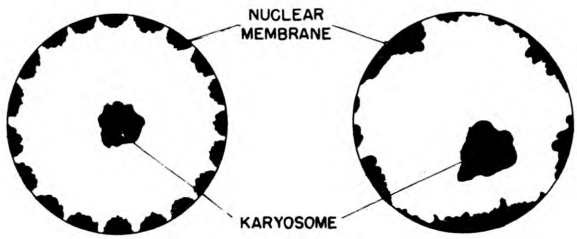
The Endolimax nucleus has a relatively large karyosome and no chromatin granules on the nuclear membrane. In optical section it resembles somewhat an eye, the karyosome being the pupil and the clear space between it and the nuclear membrane the white of the eye. The nucleus although essentially the same in structure in both the trophozoite and cyst is much smaller and appears dot-like in the encysted form.

The Iodamoeba nucleus is similar to that of Endolimax. The karyosome is usually larger and between it and the nuclear membrane is a layer of small granules. These are difficult to demonstrate, and when not seen, the Iodamoeba nucleus may not be distinguishable from the Endolimax nucleus. In the cysts, the karyosome usually lies eccentrically against the nuclear membrane. The nucleus then may resemble a basket, the handle of which is formed by the nuclear membrane and the granular layer.

The Dientamoeba nucleus has a karyosome made up of granules, usually from four to six, arranged in a circular pattern. As in Endolimax and Iodamoeba, there are no chromatin granules on the nuclear membrane. In this genus, as the name implies, a significant number, from 20 to 80%, have two nuclei.

Entamoeba histolytica

Two races or strains of this ameba are recognized: a large race, cysts averaging 12 microns, and a small race, cysts averaging 8 microns. Trophozoites may be found in liquid stools or in mucus. Immature cysts are almost exclusively uninucleate.

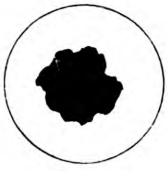


Characteristic nuclei of genus Entamoeba

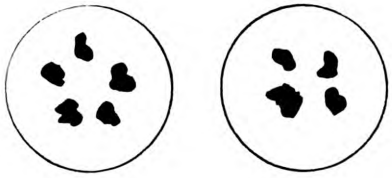
NUCLEUS OF TROPHOZOITE

NUCLEUS OF CYST

Characteristic nuclei of genus Iodamoeba



Characteristic nucleus of genus Endolimax



Characteristic nuclei of genus Dientamoeba

Fig. 17

Intestinal Protozoa
Description of protozoa, Plate I

1. A trophozoite from a nondysenteric case ("carrier"). The chromatin granules on the nuclear membrane are uniform.

2. A trophozoite in amebic dysentery, although there are no ingested red cells. The parasite is over 16 microns in length. The small karyosome is centrally placed.

3. A giant trophozoite containing ingested red cells in various stages of absorption. The nucleus is characteristic.

4. An immature uninucleate cyst (a very common type). The chromatoid matter is scattered and the outline of the glycogen vacuole is visible.

5. An immature uninucleate cyst with a large nucleus and a definite glycogen vacuole (a very common type). Note that the nucleus is not crowded by the vacuole. Chromatoid grains are scattered around the periphery of the cyst.

6. An immature cyst in which the nucleus is dividing (rare).

7. An immature cyst with two nuclei (rare). The glycogen is less definite, but the chromatoid matter is forming into bars and chunks.

8. A trinucleate cyst, one nucleus being larger than the other two (rare). A single chromatoid bar with rounded ends is present.

9. A mature quadrinucleate cyst (a very common type). The glycogen has been absorbed; the nuclei are characteristically placed. The chromatoid bar is diagnostic.

10. A mature cyst with characteristic nuclei and chromatoid bars (a very common type). Note the vacuolization.

11. A mature cyst, larger than the average for this species. The chromatoid matter has been absorbed.

12. A trophozoite, small race. The granules on the nuclear membrane are uniform and the small karyosome centrally placed.

13. An immature uninucleate cyst, small race. There is a large amount of chromatoid matter, the best diagnostic aid in the small race cysts.

14. An immature binucleate cyst with chromatoid bars. The glycogen is not so definite as seen in the large race cysts.

15. A mature quadrinucleate cyst with two chromatoid masses, one seen on end.

16. A mature quadrinucleate cyst in which the chromatoid matter has been absorbed.

Entamoeba histolytica

(Drawings from iron-hematoxylin stained smears)

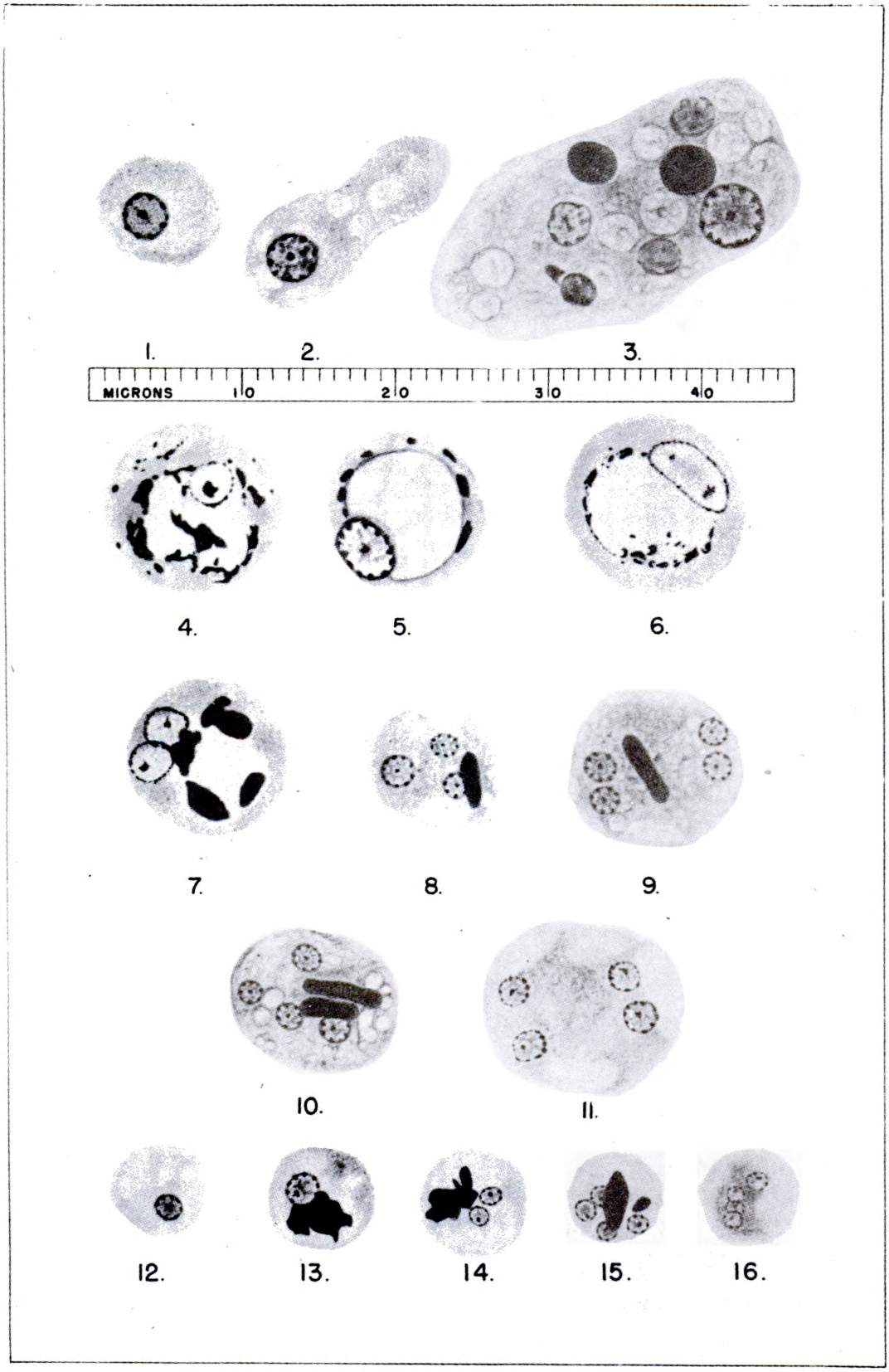
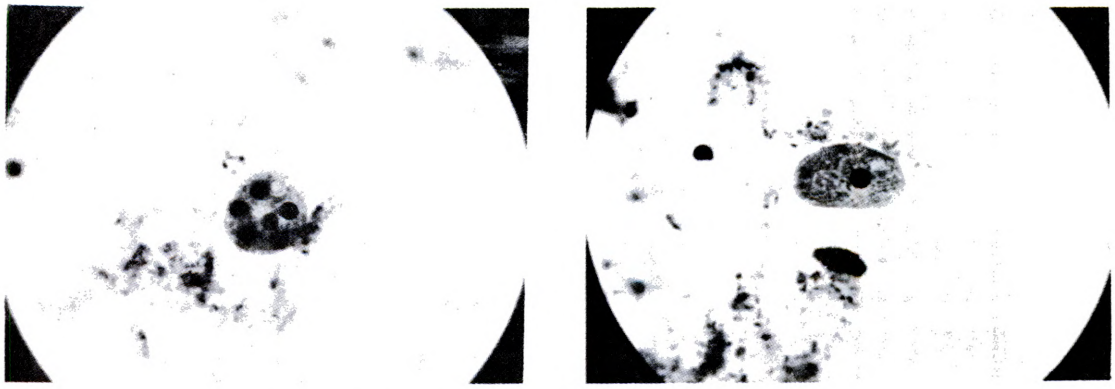


Plate I

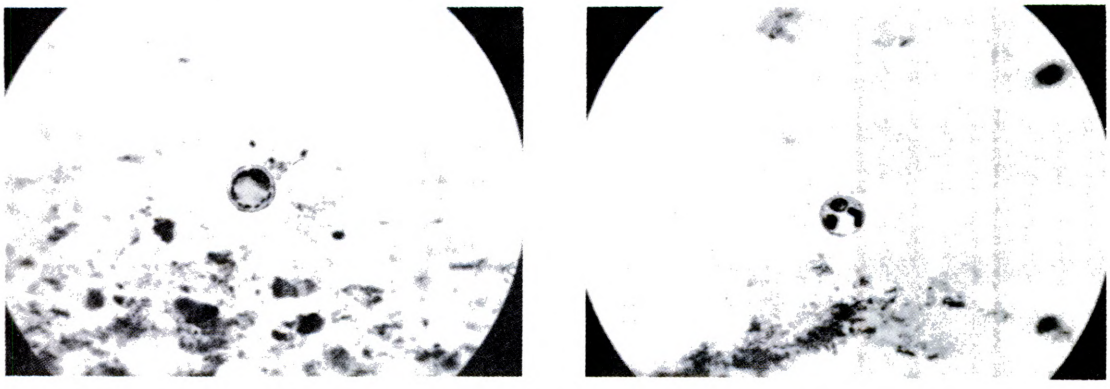
Intestinal Protozoa

Entamoeba histolytica

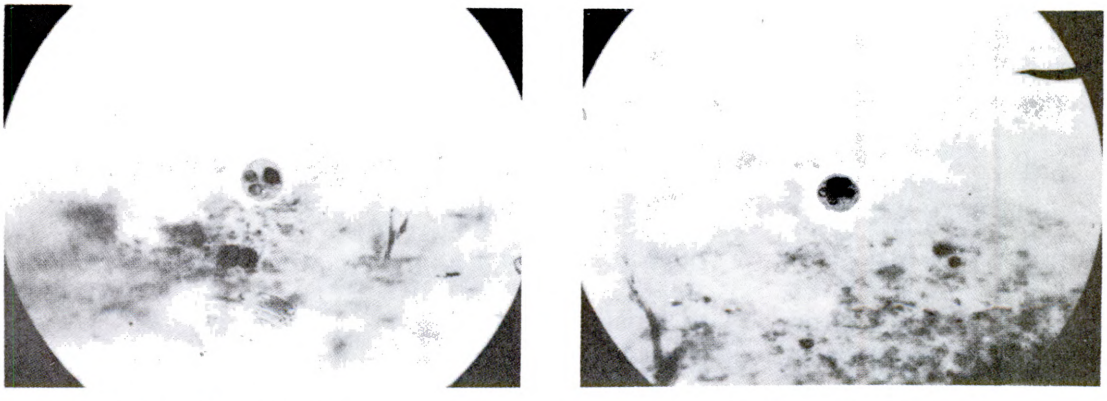
(Photomicrographs of iron-hematoxylin stained preparations)



Trophozoites with and without red blood cells



Uninucleate cysts with chromatoid material and glycogen vacuole



A binucleate cyst

A quadrinucleate cyst

With chromatoid bars

Fig. 18

Entamoeba coli

Small races of Entamoeba coli are uncommon; it is usual to find these forms significantly larger than those of the other amebas. Only the giant E. histolytica trophozoites in amebic dysentery attain or surpass those of E. coli in size. In this species, the characteristic immature cyst is binucleate, while the typical cyst of E. histolytica is uninucleate. Ninety-five per cent of the cysts of E. coli are either binucleate or eight-nucleate, the predominating number depending upon the consistency of the feces.

Description of Protozoa, Plate II

1. and 2. Two trophozoites, about 25 and 30 microns long respectively. The karyosome is large and eccentrically placed; the chromatin granules on the nuclear membrane are deposited irregularly. The endoplasm is profusely vacuolated and dirty with visible food inclusions.

3. An abnormally large immature uninucleate cyst (rare). The glycogen vacuole is large and conspicuous. Many authorities prefer to call this form a precystic stage.

4. A young immature binucleate cyst, about 17 microns in diameter (rare).

5. The characteristic immature binucleate cyst (a very common type). The nuclei assume diametric positions, crowded against the cyst wall by the glycogen vacuole. Scattered chromatoid granules surround the vacuole.

6. An immature quadrinucleate cyst (rare). The nuclei, each of which must undergo another division, are much larger and more centrally placed than in the quadrinucleate cyst of E. histolytica.

7. An immature five-nucleate cyst (rare). The three larger nuclei must undergo another division.

8. An immature six-nucleate cyst (rare). The two larger nuclei have not undergone final division. The majority of the chromatoidal material has been absorbed, not unusual in immature cysts of E. coli.

9. A mature eight-nucleate cyst (a common type). The spindle-shaped, sharply pointed chromatoid bodies are distinctive for E. coli.

10. A mature cyst with filamentous chromatoid bodies, commonly seen in this species.

11. A mature cyst with only a small speck of chromatoid matter remaining (a common type).

12. A mature cyst without chromatoid matter (a very common type).

Entamoeba coli

(Drawings from iron-hematoxylin stained smears)

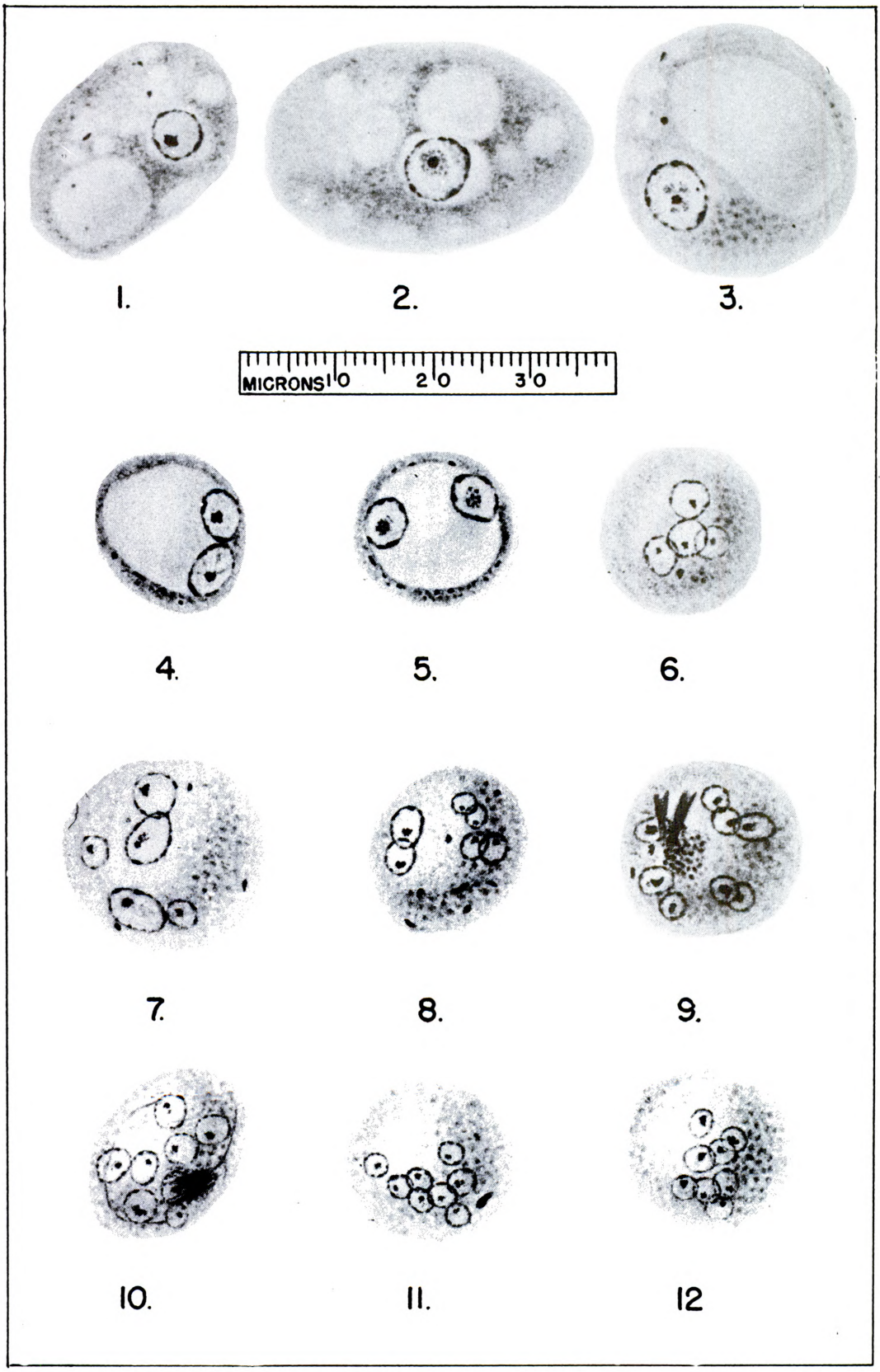
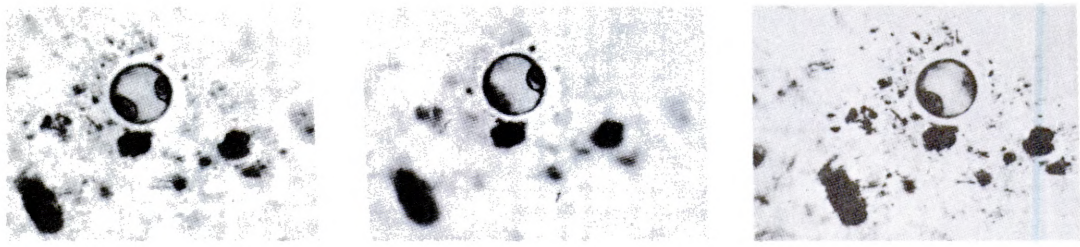


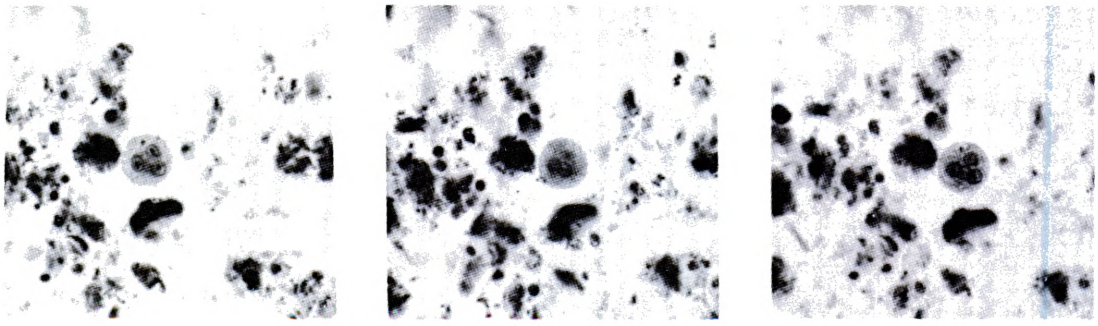
Plate II

Entamoeba coli

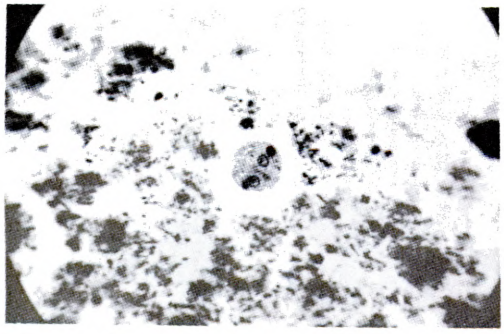
(Photomicrographs of iron-hematoxylin stained preparations;
Magn. approx. 600 x)



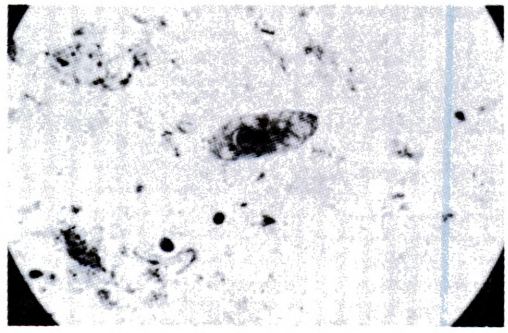
A binucleate cyst as seen in different planes and
a double exposure in these planes



A quadrinucleate cyst as seen in different planes and
a double exposure in these planes



A mature cyst
(all nuclei not visible)



A trophozoite

Fig. 19

Endolimax nana

This is a small ameba, both trophozoites and cysts being within the size range of Entamoeba histolytica small race. The cysts have no chromatoid bodies, and usually more than half of them are oval in shape.

Plate III

1. A trophozoite with a large, prominent karyosome. The nuclear membrane is not visible. The small black granules are ingested micro-organisms lying in food vacuoles.

2. An immature uninucleate cyst (rare).

3. An immature binucleate cyst (rare). The karyosome of one nucleus is split.

4. An immature trinucleate cyst (rare). Note ovoid shape.

5. A mature quadrinucleate cyst (a very common type). The nuclear membrane is faintly visible. Oval cysts seen on end will present this rounded shape.

Iodamoeba bütschlii

This ameba is generally larger than Endolimax nana, but smaller than Entamoeba histolytica large race and E. coli. The trophozoite may appear exactly like that of E. nana, but this is of little importance as neither is pathogenic. The diagnostic nucleus is brought out by staining. In addition, the cysts are of irregular shape and show a sharply defined glycogen mass and clusters of volutin granules.

Plate III

6. A trophozoite. The black granules in the endoplasm are food inclusions.

7. A young cyst with a characteristic glycogen vacuole and volutin granules (a very common type).

8. and 9. Two cysts of different size and shape (common types). They show the typical basket-like nucleus formed by the eccentric karyosome and the opposing granular layer. Glycogen vacuoles and clusters of volutin granules are present in both.

Dientamoeba fragilis

This ameba has a wide range in size, only trophozoites being found. From 20 to 80% are binucleate forms.

Plate III

10. and 11. Uninucleate trophozoites. The karyosomes are made up of distinct granules, one in triangular and one in circular pattern.

12. and 13. Binucleate trophozoites. The nuclear membrane is visible in one.

Endolimax nana, Iodamoeba bütschlii, Dientamoeba fragilis,
and Entamoeba gingivalis
(Drawings from iron-hematoxylin stained smears)

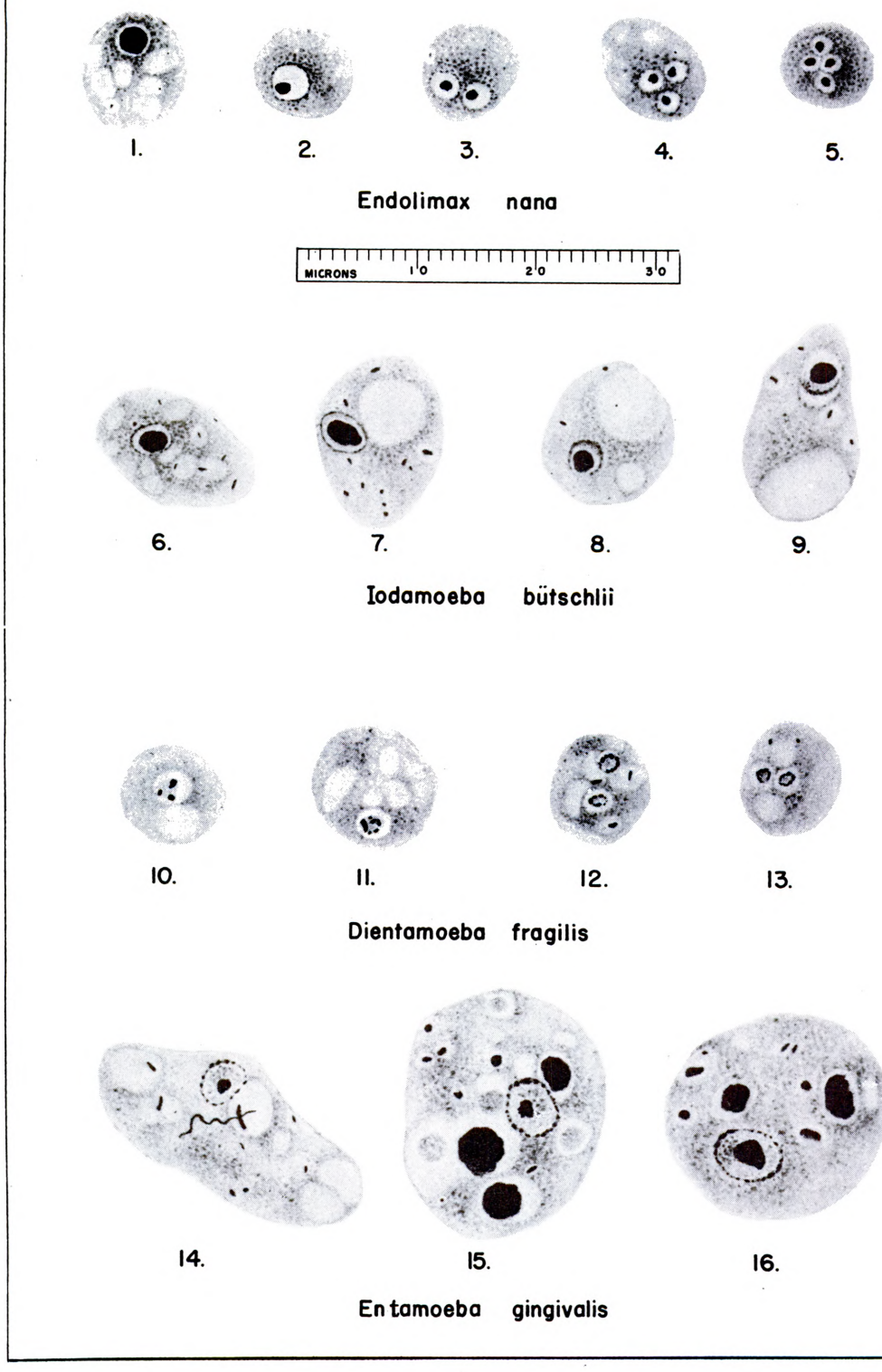
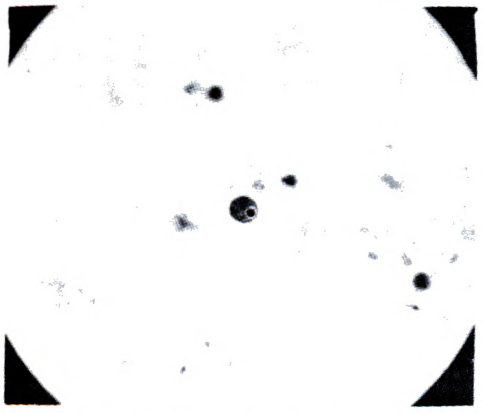


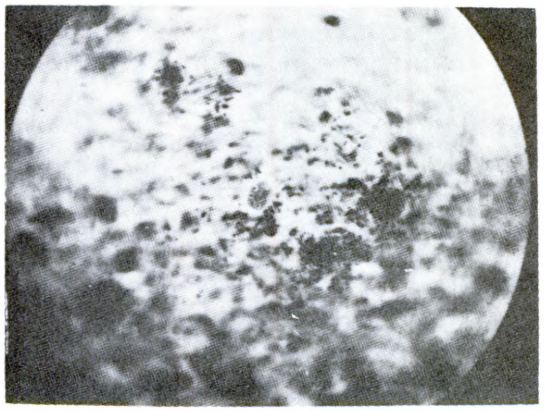
Plate III

Endolimax nana, Iodamoeba bütschlii, and Dientamoeba fragilis

(Photomicrographs of iron-hematoxylin stained preparations;
(Magn. approx. 550 x)

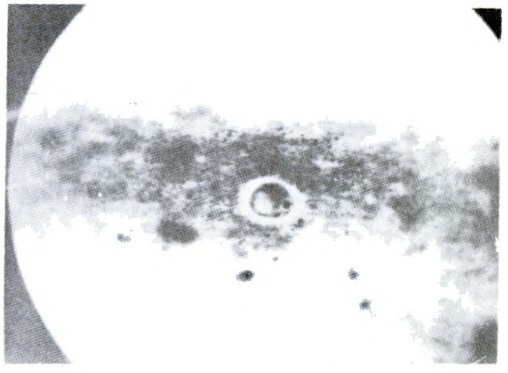


E. nana trophozoite

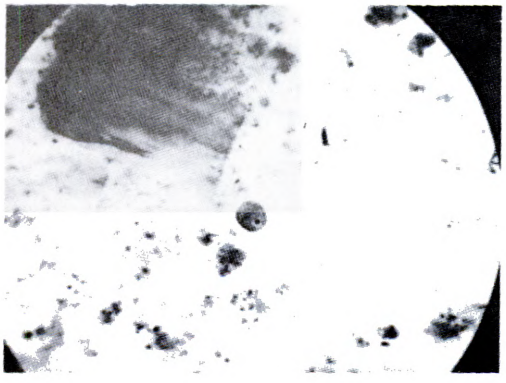


E. nana cyst

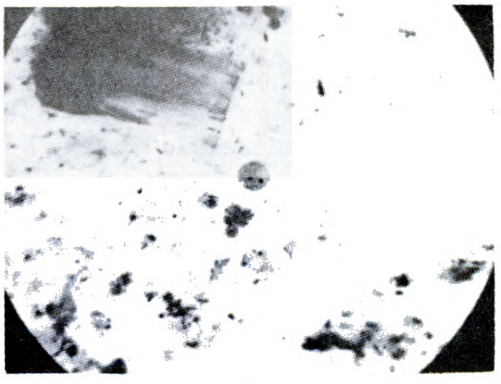
I. bütschlii trophozoite



I. bütschlii cyst



D. fragilis, uninucleate trophozoite



D. fragilis, binucleate trophozoite

Fig. 20

Entamoeba gingivalis

This ameba is related to the intestinal forms but occurs in the oral cavity. Only trophozoites are found. The nucleus is typical of the genus, more closely resembling that of Entamoeba coli. The endoplasm is filled with food vacuoles containing dark-staining bodies from ingested food material.

Plate III.

14, 15, 16. Trophozoites.

The Flagellates

Giardia lamblia - This pear-shaped flagellate has a very distinctive appearance. The organelles are in pairs and arranged symmetrically. The trophozoites (1 and 2) are more complete than those usually seen; flagella may or may not be visible, Plate IV. All have two nuclei. Cysts (3 and 4) have four nuclei arranged in pairs at the anterior pole. The fibrils and curving rods, parts of axostyles and flagella, may resemble the lacing of a football.

Chilomastix mesnili - The body of the trophozoite (5) appears as a long cone; the spiral groove is often visible. The nucleus is vesicular and contains a small karyosome. Flagella are not always seen. The lemon-shaped cyst (6) has the wall at one end thickened into a nipple-like structure. The nucleus resembles that of the trophozoite. Visible fibrils may be described as assuming the shape of a safety pin.

Trichomonas hominis and (T. vaginalis) - These two trichomonads are very similar, showing only minor differences in shape and morphology. They often stain poorly, making it difficult to recognize the undulating membrane and the number of flagella. Only trophozoites occur. (T. vaginalis is associated with the urogenital system and is likely to be found in vaginal or urethral discharges - see p. 55)

The Ciliate

Balantidium coli - The cilia of the trophozoites are usually visible in the stained smears. When properly oriented, the cytostome can be seen. The large nucleus with its characteristic shape is always visible; the micronucleus may be obscured. The cyst has a very thick wall; the macronucleus remains visible. The characteristic shape of the nuclus may be lost in tissue sections.

Objects Resembling Intestinal Protozoa

Blastocystis hominis is present in many stools, especially those that are mushy. Under the low magnification used for searching the wet smear, these organisms are extremely difficult to differentiate from protozoans; they may be confusing under the 4-mm. objective. The majority, however, have a more greenish tint than true cysts. They consist of a central structureless mass surrounded by a clear hyaline layer containing refractile granules; the latter are important identifying features. Even when stained, blastocysts may be misidentified since the darkly stained granules may appear like nuclei or chromatoid bodies.

Giardia lamblia, Chilomastix mesnili, Trichomonas hominis,
and Trichomonas vaginalis
(Drawings from iron-hematoxylin stained smears)

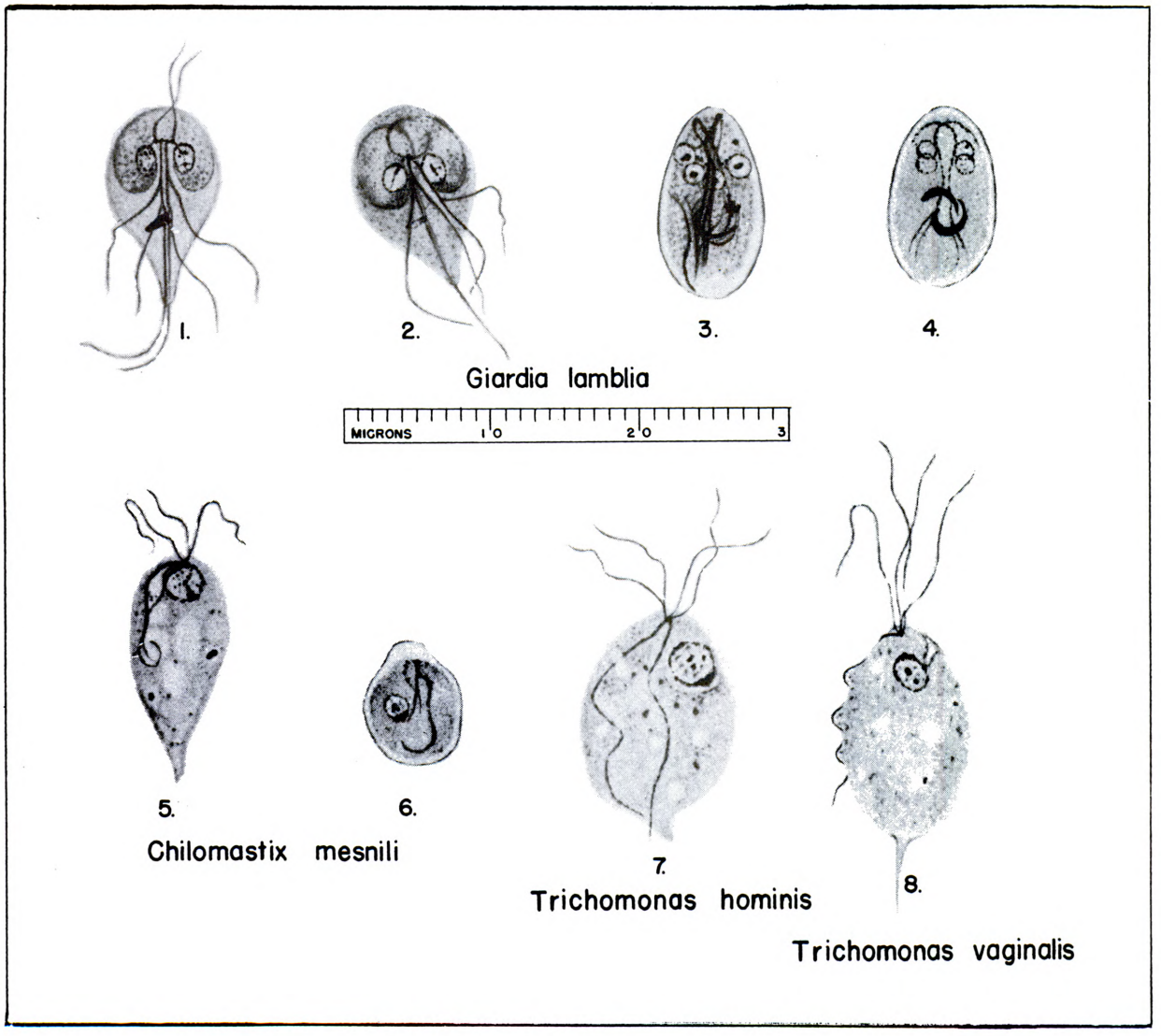
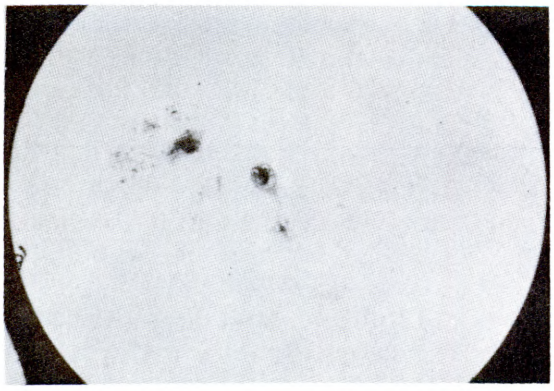
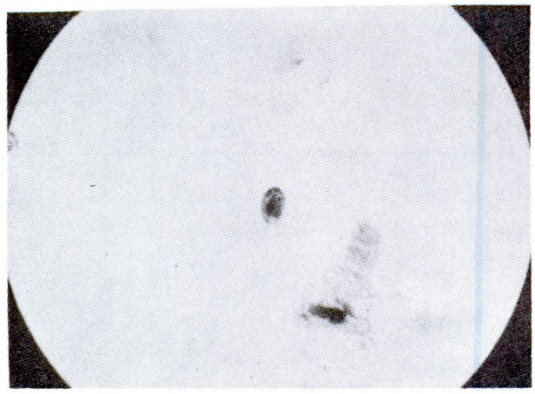


Plate IV

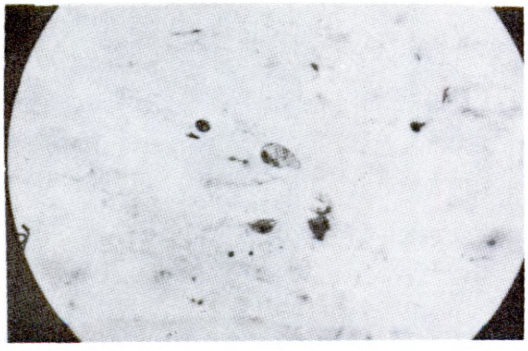
Giardia lamblia, Chilomastix mesnili, and Balantidium coli
(Photomicrographs of iron-hematoxylin stained preparations;
Magn. approx. 600 x)



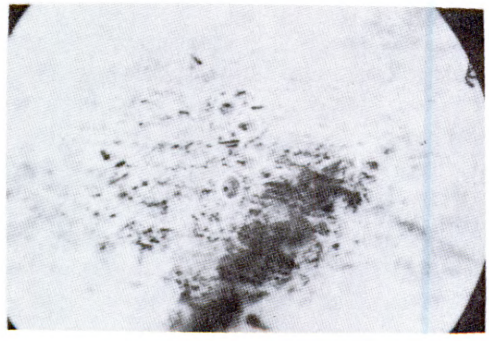
G. lamblia trophozoite



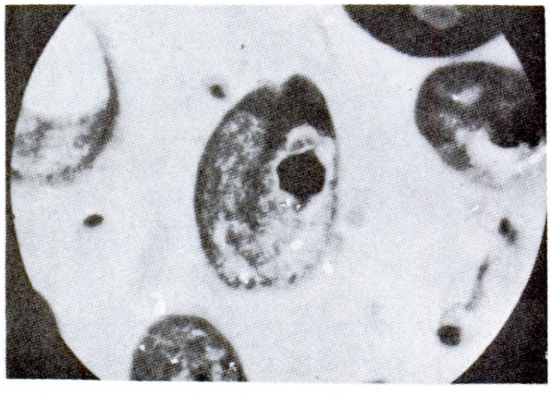
G. lamblia cyst



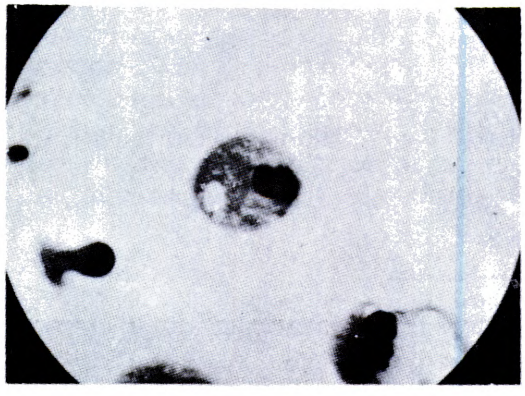
C. mesnili trophozoite



C. mesnili cyst



B. coli trophozoite



B. coli cyst

Fig. 21

In the presence of pus cells, no object should be identified as a trophozoite of Entamoeba histolytica unless it is moving. Macrophages may be especially confusing since they superficially resemble rounded-up vegetative forms of amebas. They are commonly present in the mucopurulent discharge of bacillary dysentery and may lead to a serious error in diagnosis. Polymorphonuclear and smaller pus cells are usually not mistaken for amebas in the wet smears. The segmented neutrophil with several nuclear rings may be easily mistaken for a cyst when stained. Epithelial cells are less confusing.

Yeasts occur in several forms, the commonest being a small species, 4 to 6 microns long and ovoid in shape. They may be mistaken for cysts of Endolimax nana in wet smears.

Homogeneous cysts are rounded in shape, varying in size from about 15 to 30 microns or more. While some may be colorless, most have a definite yellow tint. This color and the absence of nuclei and other organelles distinguish them from protozoans.

Charcot-Leyden crystals are frequently found in amebic dysentery but also occur in certain other diseases. They are whetstone-shaped, colorless when unstained but black in iron-hematoxylin preparations.

Starch granules that have escaped digestion may be found in the feces. Their size and refractility are the same as those of the protozoan cysts in wet smears. Under higher magnification they may be recognized by their irregular shape and surface markings. They are stained by iodine. Vegetable cells are frequently seen. These have various shapes and all have a thick cell wall. Pollen grains may have the size of cysts; they are usually brownish in color and have distinctive surface markings in various designs. Spherical clusters of minute spores of a fungus (Sphaerita) sometimes parasitize the trophozoites of the intestinal amebas.

Staining with Iron-Hematoxylin

Besides being a means of identification, the stained smear has to be used whenever permanent slides are desired. When well mounted, it remains practically unchanged for many years. At the Naval Medical School one stained slide is kept as a permanent record of each positive case of E. histolytica. There are several methods of preparing the permanent smear stained with iron-hematoxylin.

Short method:

1. Using a wooden applicator, spread a thin film of feces on a glass slide. Do it quickly to prevent drying, and then immediately immerse it in Schaudinn's solution. When the feces are dry, a small drop of water may be needed to make a uniform smear, and when there is little or no mucus in the fecal material, as in some liquid stools, a drop of egg albumin or serum will help to make the film adhere to the slide. Under all conditions, handle the slides gently in the fixing solution to prevent the smears from floating off.

Objects in Feces Resembling Protozoa
(Drawings from iron-hematoxylin stained smears)

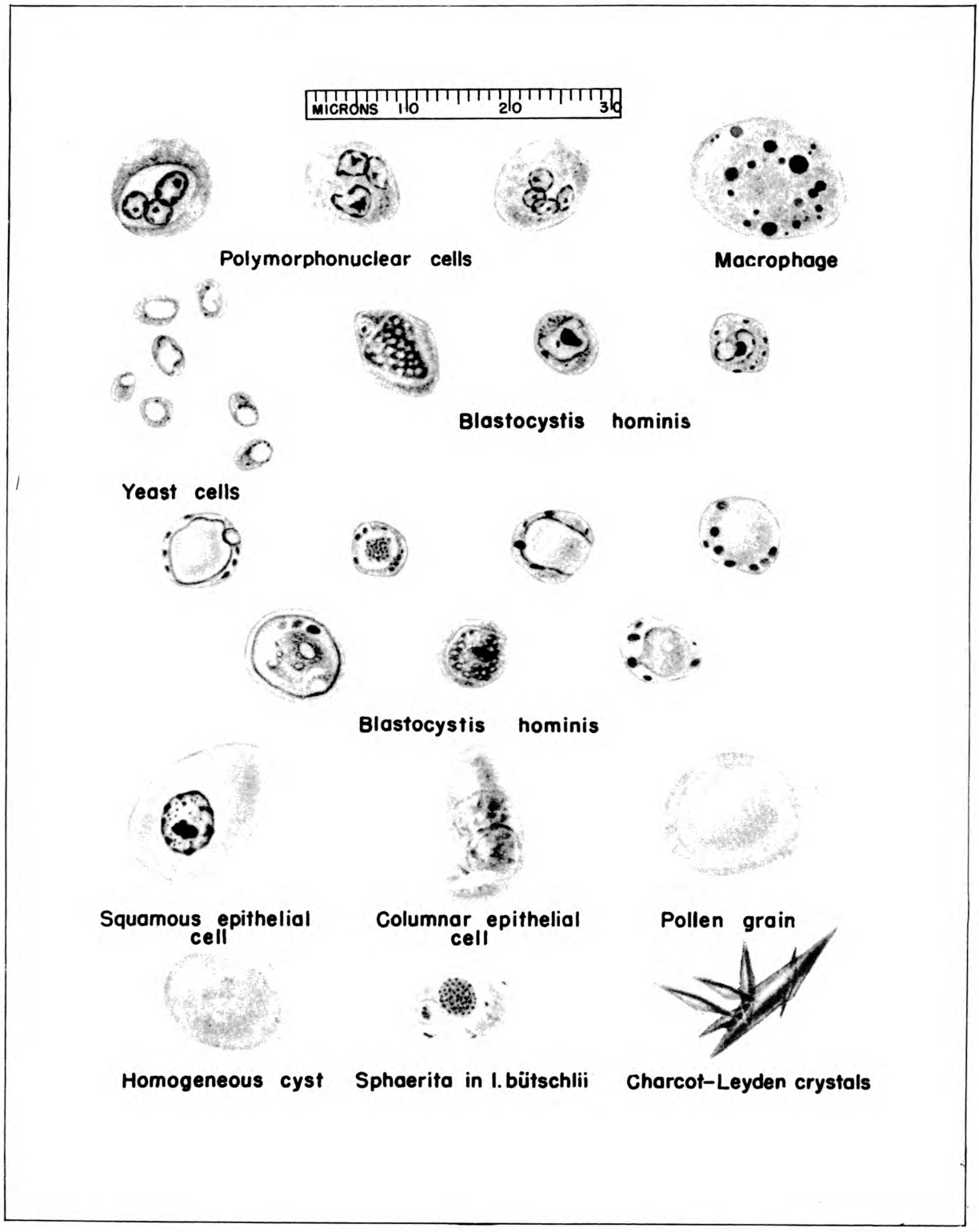


Plate V

2. Fix in Schaudinn's solution with acetic acid* . . . . . .30 min.
3. Dehydrate in 70% alcohol . . . . . .15 min.
4. Dehydrate in 95% alcohol with iodine added to a port-wine color. . 3 min.
5. Hydrate in 70% alcohol. . . . . 3 min.
6. Rinse in tap water.
7. Mordant in 4% ferric ammonium sulfate* . . . . . .15 min.
8. Rinse in tap water.
9. Stain in 0.5% hematoxylin* . . . . . .10 min.
10. Rinse in tap water.
11. Decolorize in 0.25% ferric ammonium sulfate* . . . . . .12 min.
12. Wash in running water . . . . . 1 min.
13. Dehydrate and clear in first cellosolve* . . . . . 5 min.
14. Change to second cellosolve . . . . . 5 min.
15. Mount in balsam.

*See notes on following pages:

Long method:

1. Prepare film as directed in the short method.
2. Schaudinn's fixing solution . . . . . .60 min.
3. Alcohol, 50% . . . . . 5 min.
4. Alcohol, 70% . . . . . 5 min.
5. Alcohol, 70%, with iodine to port-wine color . . . . . .10 min.
6. Alcohol, 70%, to remove iodine . . . . . .10 min.
7. Alcohol, 95% . . . . . 5 min.
8. Alcohol, 70% . . . . . 5 min.

9. Alcohol, 50%. . . . . 5 min.
10. Alcohol, 30%. . . . . 5 min.
11. Distilled water. . . . . 5 min.
12. Ferric ammonium sulfate, 4% . . . . . 6-12 hr.
13. Rinse in three changes of tap water.
14. Hematoxylin solution, 0.5% . . . . . .12-24 hr.
15. Rinse in tap water.
16. Decolorize in 1% ferric ammonium sulfate.*
17. Alcohol, 50%. . . . . 5 min.
18. Alcohol, 70%. . . . . 5 min.
19. Alcohol, 95%. . . . . 5 min.
20. Absolute alcohol. . . . . 5 min.
21. Xylol, first change. . . . . 5 min.
22. Xylol, second change . . . . . 5 min.
23. Mount in balsam.

*The ferric ammonium sulfate used for decolorizing should be freshly prepared. From time to time remove the slides from the solution and place them in water. Remove a slide from the water, dry the unsmeared side to keep the drops of water from interfering with illumination, cover the wet film with a cover slip, and examine the preparation. Use the low power to pick up the parasites and the high dry for closer study. At first the parasites will appear as fairly solid black objects which gradually become clear as the stain is removed. If the protozoan is not light enough, remove the cover slip by a quick dip in water and return the slide to the ferric solution for further differentiation. Usually this must be repeated several times. All of the slides can be transferred back and forth from ferric solution to water until the desired intensity of stain is obtained in the portions of the smear which are of average thickness. The end point of differentiation is reached when the nuclear pattern stands out clearly with all chromatin still a jet-black. The decolorization may take as long as 5 to 8 minutes, with slides usually examined at 1-minute intervals. After the film is correctly differentiated, it is transferred to a bath of running tap water for 20 minutes.

Notes on Staining and Solutions

At no time during the process of preparation, fixation, or staining can the smears be allowed to dry. Drying causes shrinkage and distortion.

The short method has certain advantages in that the diagnosis is confirmed very quickly, and the procedure can be carried out by the novice. It has some faults in that it is not permanent, causes greater distortion of protozoa, and gives a less intense stain than the long method. By increasing the time for steps (2) and (3), formerly 5 and 3 minutes respectively, some of these disadvantages have been overcome.

1. Schaudinn's solution: Prepare a saturated solution of mercuric chloride in distilled water, two parts; alcohol 95%, one part. This is kept as a stock solution. Add glacial acetic acid to a strength of 5% immediately before using. The solution should be prepared fresh each morning and renewed after staining two racks of slides (200 ml. for 40 slides). The time required for fixation may be shortened if the solution is held at 50° C.

2. Ferric ammonium sulfate solution: All ferric ammonium sulfate crystals used must be of a definite violet tint. Do not use white, yellow, or green crystals. The solution used as mordant and that used for decolorizing must be freshly prepared daily and considered exhausted after 40 slides have passed through them (for 200-ml. solution). The time required for decolorizing will vary somewhat with each batch of stain and the thickness of the smears.

3. Hematoxylin solution: Dissolve 1.0 gm. of certified hematoxylin in 10 ml. of 95% alcohol with the aid of gentle heat, and then add 190 ml. of distilled water. Plug the flask with cotton and allow to stand at room temperature in direct sunlight until "ripe," usually 3 to 6 weeks. When ripe, the stain has a reddish-brown color; a few drops of ripe stain added to a test tube of tap water gives a purple (not red) tint. This test with tap water should be carried out each morning on the stain solution being used. Stain should be renewed when it fails to give a good color reaction.

4. Cellosolve: When this material (ethylene glycol monoethyl ether) is not available, dehydrate with graded alcohol and xylol as given for the long method of staining. Cellosolve must be renewed after 50 to 100 slides have been dehydrated. Discard the first dish of cellosolve, replacing it with the second; use fresh cellosolve in the second dish.

5. Lugol's solution: Dissolve 10.0 gm. of potassium iodide in 50 ml. of distilled water. Add 5.0 gm. of iodine crystals and q. s. to 100 ml.

VIII. Detection and Identification of Intestinal Protozoa Using the MIF (M -erthilate, I -odine, F -ormalin) Technic and the Rapid Permanent Mount Stain Technic (2 1/2 minute technic)

Use of Microscope

With MIF use as described for smears in isotonic solutions and with the 2 1/2 minute technic follow procedure employed for haematoxylin smears.

MIF Technic (Sapero and Lawless, 1953)*

Purpose of Method

The purpose of this method is fourfold: (1) allows fixation and staining of parasites by the direct smear method; (2) allows for vial preservation of specimen which may be used for permanent record or for repeated studies, e.g., student or class use; (3) provides a means for simple convenient collection and simultaneous fixation and staining of freshly passed fecal specimens in the home or on the ward; and (4) it is an excellent method for use in parasite surveys where it may not be feasible to have equipment and certain materials in the field.

Solutions and Technics

Either of two procedures may be followed:

a. Direct smear preparation

(1) In a Kahn tube place 1 ml. of stain-fixative (MF) solution. This consists of 0.125 ml. of formaldehyde solution (USP) and 0.775 ml. tincture of merthiolate (No. 99 Lilly, 1:1,000). To this MF solution add 0.10 ml. of freshly prepared Lugol's solution 5 per cent (Merck Index). This MIF mixture is adequate for 25 fecal examinations. (2) With eye dropper place one drop of distilled water and a drop of MIF solution on slide and add feces to make a wet smear as described earlier for the regular direct saline smear.

b. Vial preparation for collection and preservation of fecal specimens on the ward, in the home or for transmittal to the laboratory.

(1) Prepare a stable stock solution of "MF". The following is a convenient quantity for the average laboratory; 250 ml. distilled water; 200 ml. tincture merthiolate (No. 99 Lilly, 1:1,000); 25 ml. formaldehyde (USP) and 5 ml. glycerine to make a total of 480 ml. of stock "MF". Store in brown bottle.

Prepare a 5 per cent Lugol's solution. (Must be fresh)

(2) Use screw cap vials to prevent evaporation if specimen is to be retained for future use or a Kahn tube as a temporary container for use with fecal specimens in the laboratory. Feces, "MF" stock solution and Lugol's solution may be placed in vials in proportion as follows:

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*Sapero, J.J. and Lawless, D.K., 1953: The "MIF" Stain-Preservation Technic for the Identification of Intestinal Protozoa; Am. Jour. Trop. Med. & Hyg. 2:613-619.

Vial Size	Quantity of feces	"MF" solution	Lugol's Solution
Small (approx. 5 ml.)	0.25 gm.	2.35 ml.	0.15 ml.
Medium (" 10 ml.)	0.50 gm.	4.70 ml.	0.30 ml.
Large (" 15 ml.)	1.00 gm.	9.40 ml.	0.60 ml.

Preferably Lugol's solution should be fresh (never more than 3 weeks of age) and should never be combined with "MF" stock solution until just prior to placing fecal specimen in vial or Kahn tube. Earlier addition of iodine (Lugol's) to "MF" stock solution may result in precipitation of the mixture.

The use of a small kit consisting of two screw cap vials placed in an appropriate size rectangular pill box with simple instructions has been very satisfactory for collection of fecal specimens from patients in hospital wards or for collection at home thus obviating a special trip to the hospital or clinic.

The proper volume of "MF" solution is measured into one vial and fresh Lugol's into the other vial. Mimeographed instructions with the kit state specifically that "the vial with the red (MF) solution should be poured into the vial with the brown iodine (Lugol's) solution and mixed immediately before use. An appropriate quantity of feces (depending upon size of vial and volume of MIF) is added to the vial with the MIF solution and must be thoroughly stirred with an applicator stick" -- which for convenience accompanies the kit. Vial with MIF and feces mixture should be capped and the entire kit with name of donor or patient affixed returned to the proper laboratory.

The MIF technic may be conveniently used for fixation and staining of materials taken at time of proctoscopic or sigmoidoscopic examination of patient. The collector of the sample should be encouraged to aspirate several ml. of fluid and fecal matter if feasible to do so. Just a swab will not suffice. When placed in a vial with MIF the sample should be no less than 1/5 the volume of the MIF solution. If smaller quantities (or proportions) are used the material intended for study may be "burned out" or over stained and thus rendered less desirable for examination and accurate evaluation by the laboratory technician.

In survey work the MIF technic is especially useful since it allows collection and preservation of freshly passed fecal specimens at the site of collection, employing a minimum of materials, equipment and effort. Cardboard boxes each containing 50 vials (15ml. capacity) separated by partitions to avoid breakage has been found convenient. The appropriate quantity of "MF" stock solution (9.4 ml. for 15 ml. vial) is pipetted into vials prior to departure from the laboratory. Freshly prepared Lugol's solution is pipetted from a stock bottle with a marked eye dropper type pipette just prior to introduction of fecal sample. Screw cap vials allow safe storage for several months. Even screw cap vials should be sealed with paraffin or other materials to reduce evaporation of solution if specimens are to be kept for long periods.

Technic

Examination for parasites is simple. Remove one or two drops of mixed fluid and feces from the sedimented surface layer and make a cover slip preparation. For prolonged study the cover slip may be ringed with petrolatum or other sealing materials to reduce evaporation. Staining, whether in the direct smear or from a vial preparation, consists of an initial iodine phase as seen in the ordinary iodine preparation except for the fact that both cysts and trophozoites are stained, and a subsequent eosin phase which gradually replaces the iodine. Reversal to the iodine phase may be accomplished by addition of fresh Lugol's solution to the specimen.

Trophozoite forms stain immediately whereas cysts stain more slowly. In the trophozoites of Entamoeba histolytica from cases of amebic dysentery red blood cells are clearly defined and even more readily recognized than in fresh saline preparations. In general the staining of protozoa is such as to allow identification of species using criteria applicable to a study of haematoxylin stained smears.

Rapid Permanent Mount Stain Technic

(Known also as the 2 1/2 minute or Lawless* Permanent Mount Stain)

Purpose of Method

This method provides a simple means for immediate fixation and quality differential stain of specimens which may be examined at once or retained as permanent mounts.

Solutions

(1) Schaudinn's standard fixative solution (see page 53 for preparation)

(2) Permanent mount stain

For preparation of 1000 ml. of fixing and staining solution:

acetone	50 ml.
glacial acetic acid	50 ml.
formaldehyde solution (USP)	10 ml.
Schaudinn's solution ((1) above)	890 ml.

To this solution add 2.5 grams of acid fuchsin and 1.0 grams of fast green (FCF). After thorough mixing the solution is ready for use. It should be stored in tightly stoppered brown bottle. This solution remains stable.

Technic

(1) Using toothpick or applicator stick smear a thin layer of feces on a clean glass micro slide as recommended for permanent mount preparations with haematoxylin. Fecal smear must not be allowed to dry.

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*Lawless, D.K., 1953: A Rapid Permanent-Mount Stain Technic for the Diagnosis of the Intestinal Protozoa: Am. Jour. Trop. Med. and Hyg., 2:1137-1138, or see Faust, E.C., 1955: Animal Agents and Vectors of Human Disease, p. 578.

(2) With medicine dropper or glass stoppered dropping bottle immediately cover all parts of the fecal smear with the prepared staining and fixing solution.

(3) Gently heat the stain covered smear over an alcohol lamp or laboratory burner until steam is first observed. Two or three slow passes of the slide through a flame will usually suffice. Do not boil and do not allow the staining solution to flame.

(4) Immediately wash gently with tap water.

(5) Place in 50 per cent and 70 per cent alcohol for 30 seconds each, 95 per cent and absolute alcohol for 15 seconds each and then clear in xylol for one minute.

(6) Mount in Canada balsam. Preparation is now ready for examination.

Upon examination the staining characteristics of the rapid permanent stain are similar to those seen in smears prepared with haematoxylin and the same criteria are employed for identification of the intestinal protozoa. Nuclear detail and chromatoid substance are clear and both cysts and trophozoites of all species stain well.

IX. Concentration Method

Craig and Faust (1951) and Faust (1955) have described several methods for detecting the cysts of the intestinal amebas. Following these procedures increases the total number of positive stools found and facilitates diagnosis when the organisms are scarce. One of the methods (zinc sulfate centrifugal-flotation of Faust, et al. 1938, 1939) given below concentrates the ova of the common intestinal helminths as well as the cysts, reducing the usual search for intestinal parasites to one procedure. It is time consuming.

1. Mix one part of formed (cyst-bearing) stool with ten parts warm water in a glass container.

2. Strain 10 ml. of this mixture through one layer of wet cheesecloth in a glass funnel and into a suitable test tube.

3. Centrifuge this filtrate for 45 to 60 seconds at 2,500 revolutions per minute.

4. Pour off the supernatant, resuspend the sediment in distilled water, and centrifuge again; repeat until the supernatant is clear.

5. To the final sediment add 3 to 4 ml. of 33% zinc sulfate solution (specific gravity 1.180). Mix thoroughly and then fill the tube to within one-half inch of the rim with the same solution.

6. Centrifuge again for at least 90 seconds.

7. Transfer material from the surface of the liquid to a glass slide by means of a loop, stir in a drop of dilute iodine solution, add a cover glass, and examine for cysts.

X. Cultivation Method

This method is of use in the detection of large race Entamoeba histolytica and Trichomonas vaginalis. It gives poor results with small race E. histolytica and is not successful with Giardia lamblia.

Locke-Egg-Serum Medium (Boeck and Drbohlav): Wash six eggs with alcohol, break, and emulsify in 75 ml. of sterile Locke's (or Ringer's) solution. Four milliliters are placed in test tubes, slanted in an inspissator, and heated at 70° C. until the medium has solidified. The tubes are then autoclaved at 15 pounds pressure for 20 minutes. The slants are then covered with about 4 ml. of sterile Lock's solution and serum (10 to 1), and a small amount of sterile rice starch is added. After sterility tests, the cultures may be stored until used. This medium is suitable for cultivating parasitic amebas.

INCIDENCE OF INTESTINAL PROTOZOA

The following table is from the report of a survey of 1,021 men, U. S. Navy, published in the U.S. Naval Medical Bulletin, April 1939. Repeated examinations

were made to obtain accurate figures which suggest the expected incidence of intestinal protozoa in the year 1939. More recent figures for incidence of intestinal protozoa in Naval personnel are much lower indicating possibly a reduction due to the general use of anti-biotics. Incidences cited for 1953 and 1954 and 1958 are based upon examinations made at the Naval Hospital at Bethesda.

Protozoa	Percentage Infection				
	1939	1953	1954	1958	1962
E. histolytica	11.6	1.7	0.88	0.29	0.52
E. coli	25.6	3.5	4.0	1.68	1.34
E. nana	22.7	6.2	4.9	3.13	3.10
I. bütschlii	4.1	0.2	0.2	0.06	0.04
D. fragilis	6.5	0	0	0.17	0.14
G. lamblia	7.2	2.3	2.3	1.33	1.39
T. hominis	1.3	0	0.3	0	0.14
C. mesnili	1.5	0.4	0.03	0.17	0.04

SPECIES RELATED TO INTESTINAL PROTOZOA AND LIVING IN MAN

Entamoeba gingivalis (Plate III, 14)

This ameba lives in the human mouth. Some observers have incriminated it as the cause of pyorrhea alveolaris, It is generally considered a harmless parasite which thrives in the presence of gingival infection. It resembles E. histolytica and E. coli. The food vacuoles may contain dark-staining bodies, believed to be nuclei from ingested pus cells. No cysts are formed, the trophozoites being transmitted by direct contact.

Trichomonas tenax (T. buccalis, T. elongata)

This is also a parasite of the human mouth. It can not be distinguished morphologically from T. hominis. It is most commonly found in persons with gingivitis. No cysts have been found.

Trichomonas vaginalis (Plate IV, 8)

This flagellate inhabits the vagina. It has also been found in the urinary tracts of both men and women. It has the same morphological structure as T. hominis and T. tenax. No cysts have been found. It is usually considered a pathogen which may cause vaginitis and may be associated with a non-specific urethritis. Transmission occurs through direct transfer of the trophozoites.

MALARIA PARASITES

DEFINITIONS

Schizogony - asexual multiplication by fission (in man).

Pre-erythrocytic cycle - development of parasite prior to entry into red blood cells

Erythrocytic cycle - stages of development as seen within the red blood cells

Exo-erythrocytic cycle - stages of development of parasite which take place in the reticulo-endothelial and other tissue cells. In this stage the malarial parasites contain no pigment.

Trophozoite - the vegetative or feeding stage of the parasite (in man).

Schizont - a form developed from the trophozoite in which the nucleus has divided or split (in man)

Merozoite - one of the cells resulting from division of a mature schizont (in man).

Gametocyte - a sexually differentiated cell capable of producing gametes (passed from man to mosquito).

Macro - meaning large, the prefix assigned to the female cell.

Micro - meaning small, the prefix assigned to the male cell.

Gamete - the sex cell resulting from the maturation of a gametocyte (in mosquito).

Zygote - the cell resulting from the union of two gametes (in mosquito).

Ookinete - the vermicular zygote (in mosquito).

Sporogony - sexual reproduction by the development of spores (in mosquito).

Oöcyst - the encysted form of the oökinete (in mosquito).

Sporozoite - the form resulting from the division of the oöcyst (passed from mosquito to man.

CLASSIFICATION

Phylum: Protozoa
Class: Sporozoa
Order: Haemosporidia
Family: Plasmodiidae
Genus: Plasmodium
Species in man: vivax, malariae, falciparum, ovale, knowlesi, cynomolgi, brazilianum

Other related genera and species are found in various members of the animal kingdom. In general, it may be said that plasmodia are host-specific: human malaria has not been transmitted to animals, and, with the exception of Plasmodium knowlesi, P. cynomolgi and P. brazilianum, animal malaria has never been successfully transmitted to man.

Malaria Parasites

PARASITES RELATED TO HUMAN MALARIA

Order: Haemosporidia

Family: Babesiidae

Genus: Babesia - in cattle, sheep, horses and other animals.

Family: Haemoproteidae

Genus: Hemoproteus in birds and cold-blooded vertebrates. Parasites of the genus Hemoproteus live wrapped around the red blood cells; they produce pigments.

Genus: Leucocytozoon - in birds. Species of Leucocytozoon develop in cells that have no hemoglobin and do not produce pigment.

Family: Plasmodiidae

Genus: Plasmodium

Species:

In monkeys:	In birds:
kochi	praecox
brazilianum	cathemerium
knowlesi	gallinaceum
cyomolgi	lophurae
inui	
richenowi	

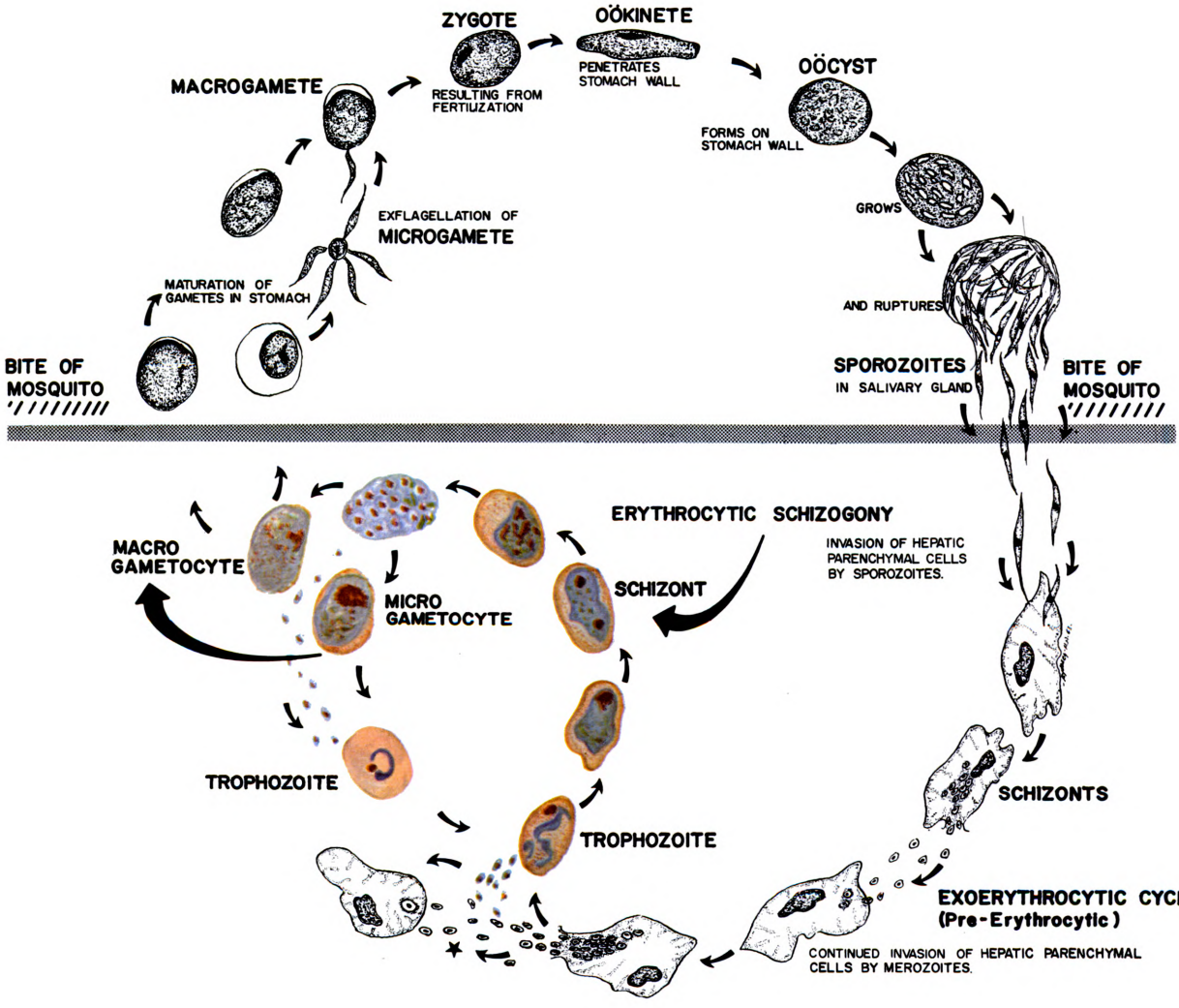
Other species are described in the bat, rat, buffalo, horse, dog, and lizard. Information in regard to the distribution of these parasites is far from complete.

LIFE CYCLES

In the life cycles of Sporozoa there is an alternation of generations - a sexual cycle, sporogony, during which multiplication occurs after union of the two sex cells; and an asexual cycle, schizogony, during which there is multiplication without previous fertilization. In some Sporozoa both cycles occur in the same host. Parasites of the Order Haemosporidia have adapted themselves to a life in the blood cells. For members of the Family Plasmodiidae, sporogony takes place in mosquitoes and schizogony in the reticulo-endothelial and tissue cells (i.e. pre - and exo erythrocytic stages) and in the red blood cells (erythrocytic stage) of vertebrates. Details given apply to the human parasites.

LIFE CYCLE OF MALARIAL PARASITES

SEXUAL CYCLE IN MOSQUITO (SPOROGONY)



ASEXUAL CYCLE IN MAN (SCHIZOGONY)

* NOTE: EXOERYTHROCYTIC CYCLE AFTER INVASION OF RED BLOOD CELLS POSTULATED IN SOME HUMAN INFECTIONS.

FIG 22

Sexual Cycle (sporogony) - in the Mosquito

When a person who is a carrier of malaria is bitten by a female anopheline mosquito, parasites are taken up with the blood. The mosquito becomes infected if both sex forms of the Plasmodium were present in adequate numbers. The ingested male and female gametocytes become altered in the stomach of the mosquito and are known as gametes. The microgametocyte undergoes a development known as exflagellation during which its nucleus divides, each part taking a filament of cytoplasm. These are microgametes (male gamete) which break away and seek out the female gamete. The macrogametocyte (female gamete) prepares for fertilization by extruding part of its chromatin. The microgamete penetrates this macrogamete and the chromatin of the two fuses. The zygote (fertilized cell), thus formed, becomes elongate and vermiform, some 12 to 24 hours after the mosquito has ingested the blood. It is moved through the stomach wall of the mosquito and forms the oöcyst between the epithelium and the muscular layer. This requires about 40 hours. The oöcyst grows and undergoes reorganization. In 4 or 5 days, thousands of sporozoites have developed in each oöcyst - sporogony. When fully developed, the oöcyst ruptures and releases the sporozoites into the body cavity of the mosquito. These migrate to the tissues, many reaching the salivary glands, and the mosquito is then infective.

The approximate time required for completion of the sexual cycle, or extrinsic incubation period, in the three well-known species under optimum conditions is given below. Both humidity and temperature are important factors, high humidity being necessary. Below 15° C. sporogony is inhibited. Once sporozoites have matured, they will survive at lower temperatures. Although mosquitoes have been found to be infective for 3 months, it is thought that that the period of infectiveness is somewhat shorter. Those that live unusually long may become noninfective. A mosquito may carry more than one species of malaria at the same time or may become reinfected. There may be few or many oöcysts developing at the same time. The asexual forms ingested by the mosquito are destroyed.

Species	Length of Sexual Cycle
P. vivax	8-10 days at 25° C.
P. malariae	18-21 days at 22° C.
P. falciparum	10-12 days at 30° C.

Asexual Cycle (schizogony) - in Man

When an infected mosquito bites man sporozoites are introduced with its salivary fluid thus initiating the pre-erythrocytic development of the parasite. Subsequent development up to the time of actual appearance of the parasite in the red blood cells is incompletely known in the case of malaria in man. The sporozoite

gains access to the circulatory system and after an hour or so apparently invades lymphoid-macrophage cells of the reticulo-endothelial system. Cryptozoites (a type of intracellular schizont) develop as a result of parasite division. These redivide and develop into metacryptozoites. Several intermediate stages of schizonts with different characteristics may occur before the pre-erythrocytic state is terminated. These may include: (1) Microschizonts which give rise to micromerozoites similar to those observed in red blood cells. These are destined to enter erythrocytes. (2) Macroschizonts which produce macromerozoites. The latter are destined to enter tissue cells. Development in tissue cells, the exoerythrocytic cycle, may continue simultaneously with the cycle in erythrocytes.

Although descriptions of this seemingly complex development have been based in part on observations on non-human plasmodia it seems probable that the cycle as given applies to the malarias of man. It should be pointed out that just before, during, or shortly after the onset of illness parasites will be found in blood smears and the asexual forms can be studied. Note should also be made of the fact that malaria infection can be produced in man by injection of blood containing asexual forms.

The erythrocytic cycle begins when the micromerozoites enter the red blood cells. Trophozoites are the youngest forms recognized in the erythrocytes. These are composed of a nucleus and cytoplasm containing a vacuole, frequently referred to as signet-ring form. These develop and grow, producing pigment which is deposited within the parasite. Amoeboid activity varies, and the infected blood cell may be changed in size and appearance by some species. The trophozoite has reached its maximum development when the asexual cycle is approximately three-quarters completed. The nucleus now splits into daughter nuclei, the parasite being identified as a schizont - having more than one nucleus or piece of chromatin material. This splitting or division, schizogony, proceeds rather rapidly until the mature schizont has developed. The resulting segments become organized, each portion of nucleus acquiring a portion of the cytoplasm. The blood cell then ruptures and the segments or merozoites and the free pigment are released into the blood stream. Within a short time the young trophozoites may again be found in the blood cells. The complete cycle, from mature schizont to mature schizont, and the production of merozoites vary with the different species:

Species	Length of Cycle	Number of Merozoites
P. vivax	48 hours	12-24, average 16
P. malariae	72 hours	6-12, average 6
P. falciparum	36-48 hours	8-32, average 20
P. ovale	48 hours	6-12, average 8

In P. falciparum infections, only the young trophozoites are usually found in the peripheral blood. The asexual cycle is completed in the capillaries of internal

organs; older trophozoites and schizonts may be found in placental smears, by bone marrow and splenic punctures, or upon postmortem examination when they may be found in the red blood cells in the capillaries of internal organs. Older forms are occasionally found in the blood smears of cases with massive infection, indicating a grave prognosis; they have also been reported as being found more frequently in heavily infected native children and have been reported on rare occasions following the institution of therapy in acute cases.

Gametocytes

After several generations of the asexual cycle, gametocytes are developed. Their production is sometimes referred to as sporogony in man since these are the forms which start the sexual cycle in the mosquito. The gametocytes develop from merozoites which are indistinguishable from other merozoites. As they grow in the blood cells, they remain compact and do not assume the ring form with a vacuole. Young gametocytes are usually not found in the blood smears since their development in all species takes place in the blood vessels of the bone marrow and spleen. Older gametocytes resemble older trophozoites in some species, but their nuclei do not divide. There may be a "pre-gametocyte" trophozoite which divides to produce merozoites, all of which then develop into gametocytes of the same sex. Macrogametocytes are more numerous than microgametocytes. Authorities do not agree as to the time required for a gametocyte to develop from a merozoite; some believe it to be twice as long as the asexual cycle. A P. vivax gametocyte may be found in a red cell with a mature schizont, suggesting that both complete their development in the same period.

Species	Appearance of Gametocytes
P. vivax	Early - shortly after trophozoites
P. falciparum	Moderately late - about 10 days after trophozoites
P. malariae	Late - usually 30 days after trophozoites

Relationship between Life Cycle and Clinical Symptoms

The time between the bite of the mosquito and the development of symptoms in man is referred to as the clinical or intrinsic incubation period. This varies with the different species; the time required for five asexual cycles has been suggested as a minimum.

Species	Incubation Period in Man
P. vivax	12-17 days, average 14
P. malariae	21-35 days, average 28
P. falciparum	8-14 days, average 12

There are no symptoms during the early stage in man; clinical illness does not develop until parasites are present in sufficient number to produce symptoms; they may appear before parasites can be detected in the blood smears of persons highly susceptible to the infection. On the other hand, persons who have developed a high tolerance will show parasites in significant numbers without symptoms. The number of parasites required to produce symptoms also varies with the species. The expert technician using the thick film will always find parasites in P. vivax and P. malariae infections.

When the asexual cycle has been established, the parasites increase in geometrical progression at a rate dependent upon the number of merozoites in the mature schizont and the length of the asexual cycle. In the acute case they will be more numerous and more easily found on successive days. As the number increases, the symptoms become more severe and the characteristic malaria paroxysm is initiated. This sharp temperature rise, often accompanied by a chill, is preceded by the rupture of the blood cells and the release of the merozoites. All of the parasites present in the blood do not complete schizogony at the same time, but synchronous rupture of most of the schizonts occurs as infections with P. vivax or P. malariae progress. Thus, there is usually a predominating parasite form in the blood smear at any one time, with a minority of other forms. The parasites may be grouped to mature at 24-hour intervals so that two (in P. vivax) or even three (in P. malariae) forms may be most numerous. From the predominating form or forms present, the clinical cycle may be predicted. The parasites of P. falciparum show little tendency to become synchronized in their cycle with a corresponding unpredictable course. Also, P. falciparum trophozoites remain in the peripheral circulation for only 24 to 36 hours; this results in marked fluctuation in the number of parasites which may be found in repeated blood smears, and they may be difficult to find at any one time.

Infection with P. vivax is called tertian malaria because the synchronous rupture of schizonts every 48 hours produces fever on the third day. Infection with P. malariae is called quartan malaria because of its 72-hour cycle and resultant fever on the fourth day. Falciparum malaria is sometimes called subtertian because its cycle is shorter than tertian, or estivo-autumnal since the majority of cases occur in the late summer and fall. Vivax malaria is also called benign tertian because it is rarely fatal, in contrast to falciparum which is referred to as malignant tertian.

With the exception of P. falciparum, untreated malaria infections usually become limited in the severity of the paroxysms and in the number of parasites in the blood. When these parasites approximate 200,000 or more per cubic milliliter, the condition of the patient may be, or may rapidly become, very critical. Gametocytes, when present without asexual forms as often seen in P. falciparum, do not produce symptoms. They are short-lived and die unless picked up by the mosquito.

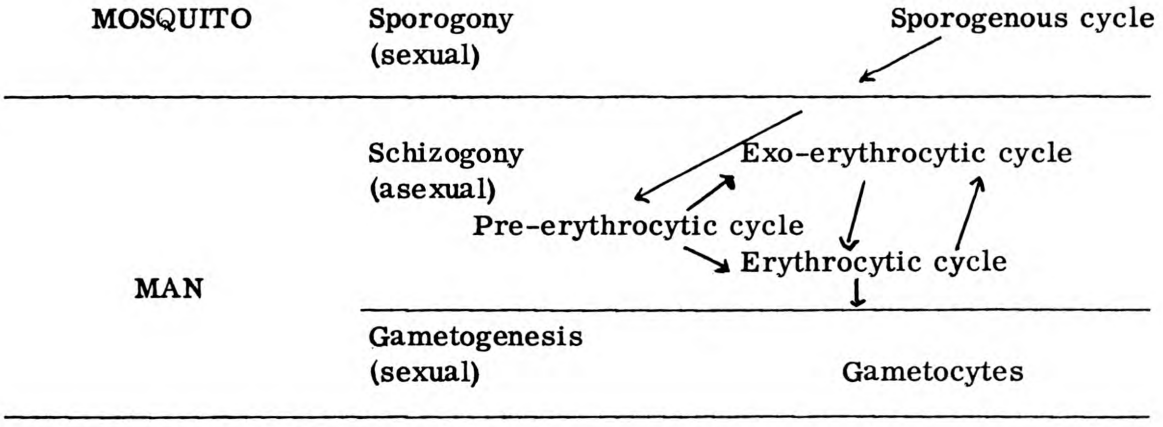
The asexual cycle repeats in the untreated case for a period of time which varies with the species involved. Then there is a spontaneous termination of the symptoms and the parasites disappear. A series of recurrences often follows, also varying with the species. Approximate or average figures are given below.

Species	Parasites per cu. ml. Average	Usual Maximum	Duration of First Attack, Untreated	Period Covered by Recurrences
P. vivax	20,000-40,000	50,000	3 weeks	2-3 years
P. malariae	5,000-10,000	20,000	6 months	7-10 years
P. falciparum	Unpredictable	No limit	11 days	2-6 months

Exo-erythrocytic development of malaria

Our knowledge of the exo-erythrocytic cycle of the malarial parasite is based primarily upon studies followed with the plasmodia of birds. However there is evidence that such a phase of development occurs in the human malarias and exo-erythrocytic stages have been observed. In the exo-erythrocytic cycle schizogonic development takes place in reticulo-endothelial and other tissue cells and the parasites contain no pigment. Apparently the exo-erythrocytic forms develop as (1) certain merozoites of the erythrocytic cycle enter tissue cells or (2) as a result of tissue cell invasion by the macromerozoites which were produced earlier during the pre-erythrocytic stages.

The scheme given below shows the relationship of different developmental stages of the malarial parasite:



LABORATORY REPORTS

The medical officer in charge of the clinical case of malaria is especially interested in the confirmation of his diagnosis. This is usually possible even though the species cannot be identified. The species diagnosis may still be made during

the next few hours after therapy has been started. This has a definite bearing on the prognosis of the case: only infections with P. falciparum are likely to cause death and only the infections with the other species are likely to cause relapses. Therefore, the laboratory report should first show the presence or absence of parasites; this should be followed by species identification, or subsequent specimens should be obtained to give a species diagnosis whenever possible.

Two additional reports are sometimes of importance - the forms of the parasites present and the relative or definite total number. The forms of the asexual cycle which are found usually have little bearing; often the clinical course will suggest the forms to be found. The presence of older forms of P. falciparum has a definite significance in the acute case. The reporting of gametocytes may have a bearing upon the control of malaria transmission, especially in P. falciparum. These forms are found in a significant number of cases when post-treatment or release examinations are made.

The number of parasites present is usually reported in two ways:

1. As the average number of parasites found per microscopic field in the thick film. This is obviously only an approximate number since the thickness of the film will vary. However, it has been found satisfactory for most purposes. The parasites may vary from 1 per 50 fields to several per 1 field. A report of 500 or more per field suggests an overwhelming infection with P. falciparum.

An alternate method is to report the ratio of parasites to white cells as seen in the thick film. This is also only an estimate since the number of white cells will vary from time to time.

2. An accurate report may be required for special purposes. When the parasite density is relatively low, this is best done by making a white blood cell count at the same time the thick film is made. The ratio of parasites to white cells is determined in the thick film and the actual number of parasites per cubic milliliter of blood is determined from this ratio and the total white count. If the parasite density is high, more accurate figures are obtained by making a red blood cell count at the same time a thin smear is made. The ratio of parasites to red cells is determined in the thin smear and the total number computed from the red count. Cases with 5% of the red cells parasitized may be considered critical, and cases with more than 20% of the cells infected rarely survive.

An alternate method employs a ruled glass slide on which a known volume of blood is made into a thick film of known dimensions. The parasites are counted much as the blood cell count is made.

DETECTION AND IDENTIFICATION OF MALARIA PARASITES IN THIN BLOOD SMEARS

A diagnosis of malaria is made or confirmed when parasites are demonstrated in a blood or tissue specimen. The technic used for blood is the same as that employed in making differential blood counts, Wright's stain being used. Giemsa's stain may be used routinely to stain both thick and thin blood smears; when used for thin smears, the blood must first be fixed by immersing the slide in methyl alcohol for a few seconds and allowing it to dry. The specimen may then be placed in the aqueous Giemsa's solution. Clean slides free from grease are required so that the blood will be evenly spread. Smears must be thin enough so that the cells will not be piled up.

The location of parasites in the thin smear is slow and tedious when they are not numerous. A negative report should not be given until the slide has been searched for 30 minutes: it is believed that symptoms may be produced by one parasite per 100,000 red cells, and it will take 30 minutes to scan that many cells. The parasites show contrast staining with the nucleus or chromatin red and the cytoplasm blue when the pH of the stain is properly controlled. Pigment is unstained and varies in color from yellow to black.

The identification of species requires a careful study of all forms, starting with the morphology as seen in the thin blood smears. When only a few young trophozoites are found, it will be difficult if not impossible to make a species diagnosis. Those of P. vivax are described as large, up to one-third the diameter of the red cell; those of P. falciparum are smallest, one-fifth the diameter of the cell. The rings of quartan are about the size of P. vivax, or slightly smaller, and appear thicker. The chromatin dot of P. vivax is described as being within the ring of cytoplasm, while that of P. falciparum is often outside the ring and is frequently double. However, P. vivax trophozoites occasionally show double chromatin dot forms. Technically these are schizonts since the chromatin mass is divided, but it is apparent from their size that the parasite is not old enough to be a normal schizont.

A diagnosis of species based upon such differential points may not be reliable and should be confirmed by subsequent smears. These young parasite forms are least diagnostic for species, and smears taken several hours after or a few hours before the paroxysm are of most value in P. vivax and P. malariae. The parasites of P. falciparum are more readily found shortly after a temperature rise, and smears repeated at intervals of 6 to 8 hours may be necessary in order to find parasites or to confirm the species diagnosis.

Often more than one parasite is found in the same red cell. This rarely occurs with P. malariae, is frequently seen with P. vivax, and is most common with P. falciparum as the number of parasites increases. Characteristic stippling of the

MORPHOLOGY OF MALARIA PARASITES IN THIN BLOOD SMEARS

	P. vivax	P. malariae	P. falciparum
Color of Cytoplasm	light blue	deep blue	blue
Color of Pigment	yellow to brown	black	brown to black
Young Trophozoites			
Form	small and large rings	small, compact rings	small, delicate rings, often marginal
Chromatin	usually one dot	large, single dot	often double dots
Pigment	none	absent or fine	none
Growing Trophozoites			
Form	irregular contour	oval or bands	rings
Chromatin	dot or thread	dot or thread	often double dots
Pigment	fine granules	coarse granules	scanty granules
Infected Blood Cell			
Appearance	enlarged, pale	normal	usually normal
Stippling	Schüffner's	none	Maurer's dots
Multiple Parasites	common	rare	very common
Growing Schizonts			rare
Form	irregular contour	compact; oval or round	rarely seen; resemble P. malariae
Chromatin	2-10 granules	2-6 granules	2-8 small clumps
Pigment	fine granules or clumps	coarse granules or clumps	fine granules or a single mass
Mature Schizonts			rare
Merozoites	12-24; in two rings or irregular	6-12; single ring or cluster; rosette or "daisy"	8-32; in two rings or irregular; small
Pigment	eccentric mass	central mass	single, dark mass

infected red cell may be observed as the parasites develop. Modified staining is sometimes necessary to bring this out. Schüffner's granules are seen in many of the cells containing P. vivax parasites; they are more often present when the slide is overstained. Similar stippling has been described with P. malariae but is usually not seen unless the pH of the stain is increased. Maurer's dots or clefts are occasionally seen in P. falciparum infections. These are darker staining, irregular in size, usually larger, and fewer in number than the Schüffner's granules.

Gametocytes

The gametocytes are of limited assistance to the technician making a species diagnosis. Those of P. falciparum are readily distinguished from all others by their sausage-like or crescent shape. Their characteristic appearance suggests ease in making this diagnosis. It must be remembered that they are produced late in the course of infection and usually will not be found during the acute illness. When P. falciparum gametocytes are found in the presence of asexual forms, a relapse is suggested. It is also possible that a mixed infection existed or that a reinfection has occurred, the asexual forms of other species causing the current illness, the gametocytes being carried over from a previous attack.

The gametocytes of the other two species, P. vivax and P. malariae, are much alike except for the larger size of the former and the coarser pigment of the latter. Young gametocytes are very much like old trophozoites, especially in quartan infection. The P. vivax trophozoites are likely to be more irregular in outline or contour, less pigmented, and more often show vacuoles. Gametocytes should be recognized so that the heavily pigmented forms found with the asexual parasites of P. vivax will not be attributed to an infection with P. malariae or diagnosed as a mixed infection.

At times it may be convenient to differentiate between the macrogametocyte and the microgametocyte of the same species. The following points are of assistance:

Microgametocyte	Macrogametocyte
Smaller in size	Larger in size
Chromatin diffuse, more pale and centrally placed	Chromatin compact, more deeply stained, often eccentric
Cytoplasm pale and often almost colorless	Cytoplasm stained a more definite blue

Gametocytes become modified in blood which is allowed to stand without clotting or drying, especially if increased carbon dioxide is present. The parasites develop bizarre shapes and exflagellation may occur. In the usual preparation the blood dries and the parasites are killed.

Key to the Species of Malaria Parasites in Thin Blood Smears

1. Only very young ring trophozoites present; no enlargement of the infected red blood cell; no stippling of the infected red blood cell. . . . . Plasmodium, species undetermined*
   Older forms of the asexual cycle or gametocytes present 2

2. Parasitized red blood cell enlarged; or, parasite with markedly amoeboid cytoplasm; or, infected red blood cell with Schüffner's stippling; or, parasite larger than normal red cell and with more than 12 nuclei. . . . . Plasmodium vivax
   Without any one or any combination of the above characteristics 3

3. Schizonts with six to twelve nuclei arranged in a "daisy-head" or rosette pattern; or, trophozoites with compact, heavily pigmented cytoplasm; or, with pigmented equatorial band trophozoites or young schizonts . . Plasmodium malariae
   Without any one or any combination of the above characteristics, and in addition, the slide must show crescent-shaped gametocytes; or, parasitized red blood cells with Maurer's dots . . . . . Plasmodium falciparum

*In such slides the technician can never be sure that any report other than "positive for malaria parasites" is correct. The commonly accepted procedure is to make such a report and request an additional smear to be made about 6 to 8 hours later. Immediately initiated treatment will have no appreciable effect in that length of time. If the parasites of the later smear show no development, a diagnosis of P. falciparum is justified. In addition, certain other features give strong evidence P. falciparum is present. These may be called percentage characteristics, i.e., they may and do occur in the other species but in much smaller numbers than in P. falciparum. An observation of one feature is interesting, two make a strong impression, and three or more almost a certainty. These characteristics are:

1. Presence of a large number of parasites and nothing but "ring" forms.

2. Multiple infection of the red blood cell.

3. Double nuclear dot trophozoites where both nuclei are round and neither appears to be a fragment.

4. Appliqué or marginal parasites.

PLASMODIUM VIVAX (Tertian Malaria) GIEMSA'S STAIN
(Plate 6)

Infected red blood cells are enlarged and blanched. Schüffner's granules are found in all but those parasitized by the youngest forms.

I. Young Trophozoites (x 2,000)
- A and B. Young ring forms (not diagnostic for species).
- C and D. Half-grown trophozoites. Note amoeboid character of cytoplasm.

II. Old Trophozoites (x 2,000)
- E, F, and G. Trophozoites with nuclear chromatin ready to subdivide. Note similarity to sexual forms and amoeboid character of cytoplasm.

III. Young Schizonts (x 2,000)
- H and I. Young schizonts showing first division of nuclear chromatin.
- J and K. Older schizonts showing from four to many subdivisions of nuclear chromatin.

IV. Half-grown Schizonts (x 2,000)
- L, M, and N. Schizonts showing seven, eight, and ten nuclear subdivisions.

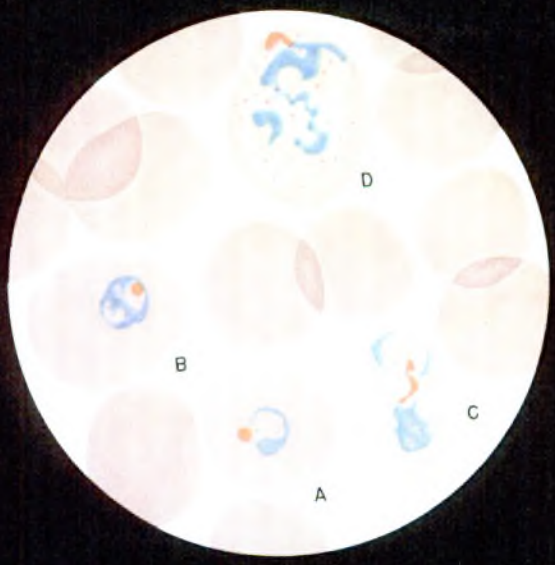
V. Mature Schizonts (x 2,000)
- O, P, and Q. Mature schizonts showing complete subdivision of nuclear chromatin and clumping of malarial pigment. Note number of merozoites and arrangement of merozoites and pigment.

VI. Gametocytes or Sexual Forms (x 2,000)
- R. Male gametocyte or microgametocyte. Note diffuse nuclear chromatin.
- S and T. Female gametocytes or macrogametocytes. Note compact chromatin and blue cytoplasm.

Approximate Age of Parasite Forms

A.	2-3 hours	H and I.	38-40 hours
B.	4-6 hours	J and K.	42-44 hours
C and D.	10-12 hours	L, M, and N.	44-46 hours
E and F.	20-30 hours	O and P.	46-48 hours
G.	30-35 hours	Q.	48 hours

PLASMODIUM VIVAX (TERTIAN MALARIA) GIEMSA STAIN



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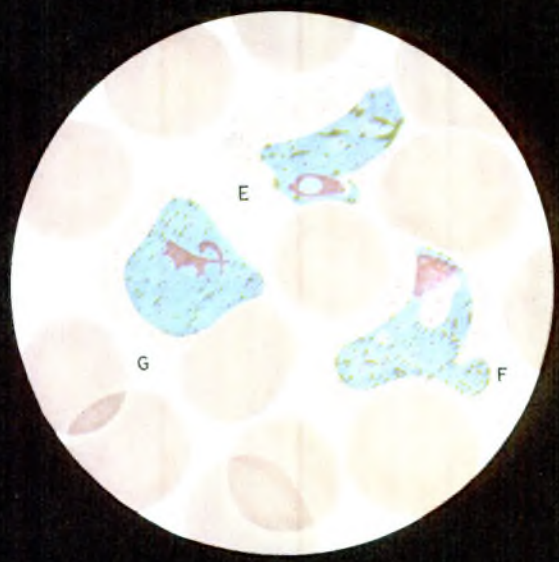

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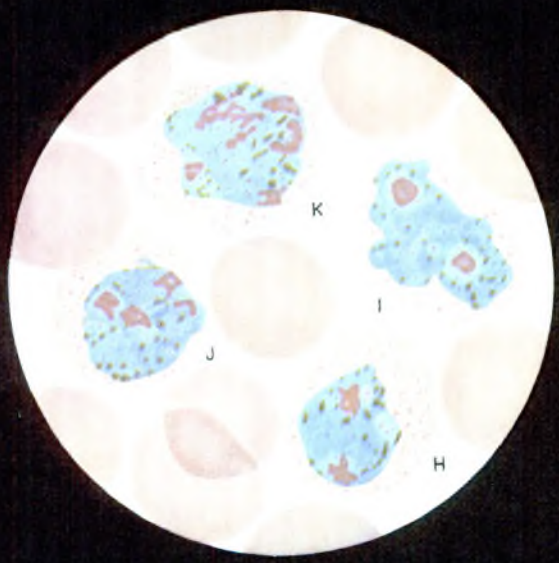

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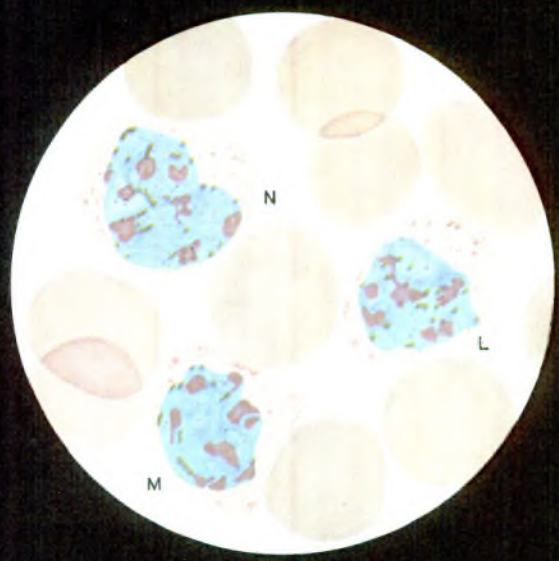
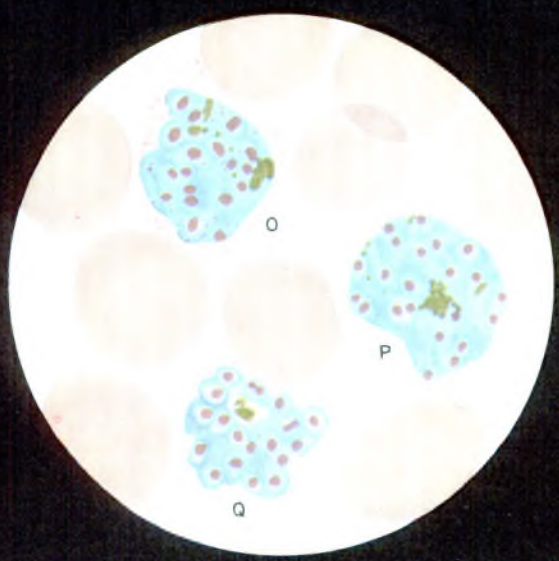


IV



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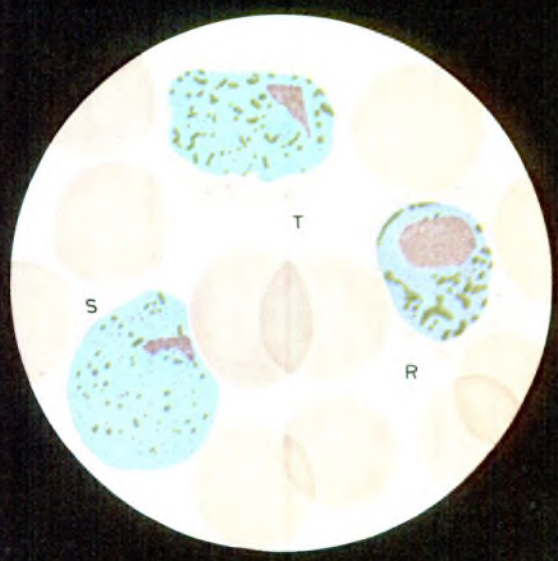
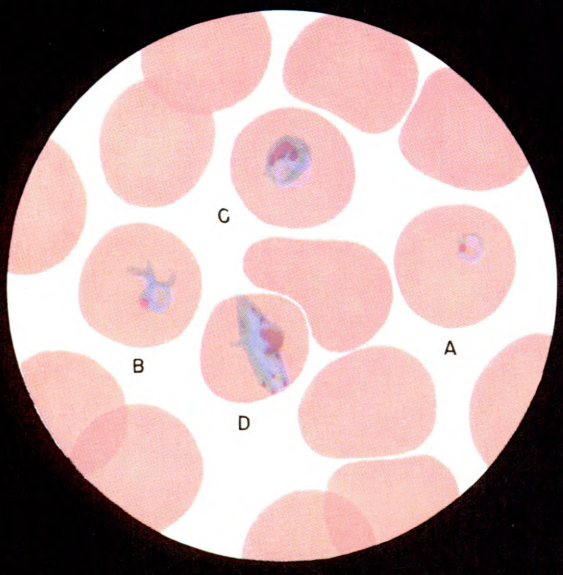


VI

PLASMODIUM MALARIAE (QUARTAN MALARIA) GIEMSA STAIN



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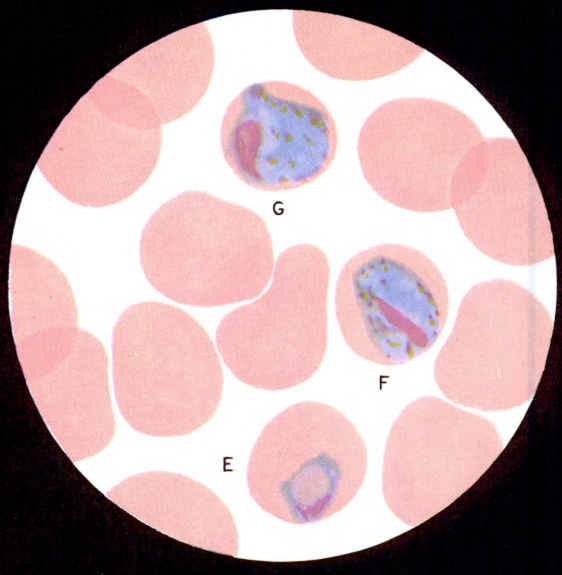

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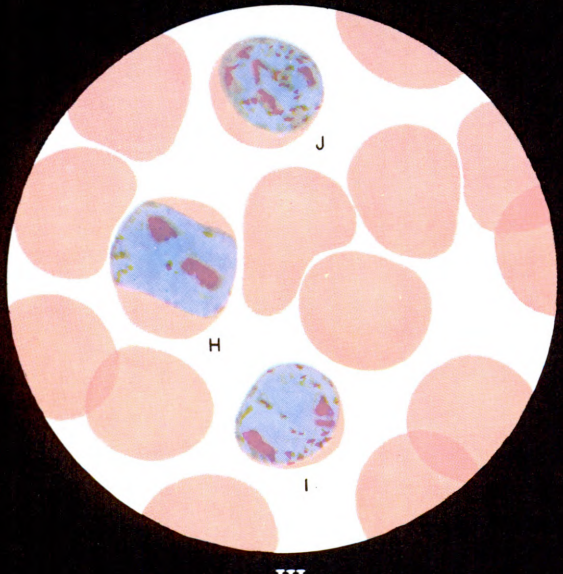

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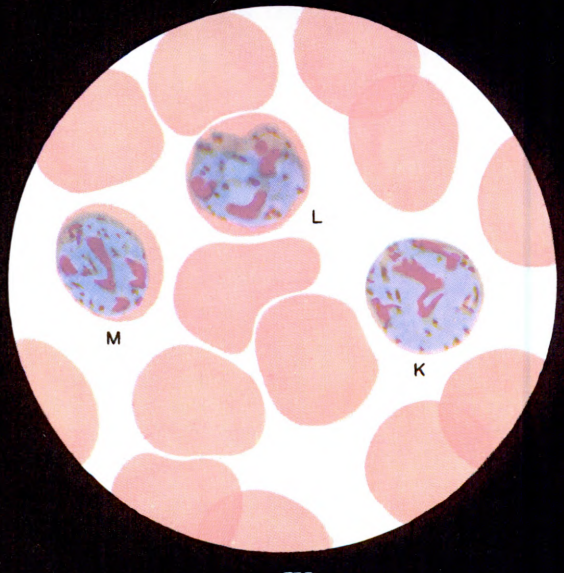

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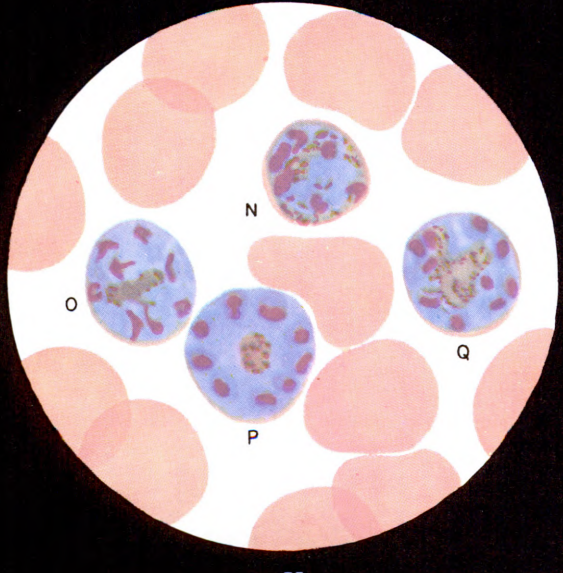

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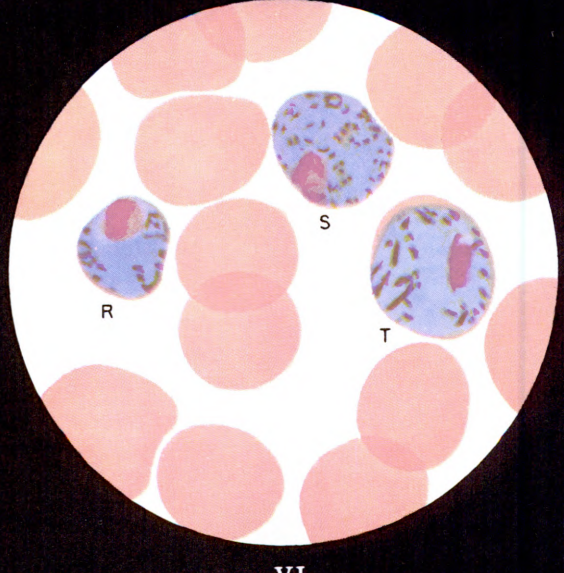


VI

NAVAL MEDICAL SCHOOL

PLASMODIUM MALARIAE (Quartan Malaria) GIEMSA'S STAIN (Plate 7)

Infected red blood cells are not enlarged or blanched. Schüffner's granules are absent.

I. Young Trophozoites (x 2,000)
- A, B, and C. Progressively older ring forms (not diagnostic for species).
- D. Band trophozoite.

II. Half-grown Trophozoites (x 2,000)
- E. Ring form (not diagnostic for species).
- F and G. Mature trophozoites. Note amount of pigment; compactness of cytoplasm.

III. Young Schizonts (x 2,000)
- H. Band schizont.
- I and J. Three- and five-nucleated schizonts. Note large amount of pigment.

IV. Half-grown Schizonts (x 2,000)
- K, L, and M. Four- to six-nucleated schizonts. Note amount of pigment.

V. Mature Schizonts (x 2,000)
- N, O, P, and Q. Eight- or ten-nucleated schizonts ready to segment and release merozoites. Note number of merozoites and arrangement of merozoites and pigment to form "daisy-head."

VI. Gametocytes or Sexual Forms (x 2,000)
- R and S. Male gametocytes or microgametocytes. Note diffuse nuclear chromatin.
- T. Female gametocyte or macrogametocyte. Note compact chromatin and blue cytoplasm.

Approximate Age of Parasite Forms

A.	3 hours	G.	50 hours
B.	10 hours	H and I.	55-60 hours
C.	15 hours	J, K, L, and M.	60-65 hours
D and E.	24 hours	N and O.	65-70 hours
F.	40 hours	P and Q.	72 hours

PLASMODIUM FALCIPARUM (Estivo-Autumnal Malaria) GIEMSA'S STAIN (Plate 8)

I. Trophozoites in Peripheral Blood (x 2,000)

- A. Young ring form (not diagnostic for species).
- B, C, and D. Young trophozoites. Note multiple infection of red blood cell, appliqué or marginal forms.
- E. Older forms. These are the oldest asexual forms of P. falciparum normally found in the peripheral blood. Note double chromatin dot in one form; Maurer's dots in cytoplasm of infected red blood cell; absence of Schüffner's dots; red blood cell not enlarged.

II. Gametocytes or Sexual Forms in Peripheral Blood (x 2,000)

- F. Male gametocyte or microgametocyte folded over and resembling slightly a microgametocyte of Plasmodium malariae.
- G. Microgametocyte. Note diffuse red chromatin.
- H. Female or macrogametocyte. Note compact nuclear chromatin and blue cytoplasm; remnant of red blood cell visible on lower margin.

III. Impression Smear of Spleen (x 2,000)

- A. Free pigment of splenic pulp.
- B. Pigment phagocytized by macrophage.
- C. Half-grown schizont in red blood cell.
- D. Parasitized red blood cells.
- E. Lymphocytes.

IV. Impression Smear of Bone Marrow (x 2,000)

- A. Free pigment granules.
- B. Nucleated red cell.
- C. Phagocytized pigment in a macrophage.
- D. Parasitized red blood cell.
- E. Eosinophile.

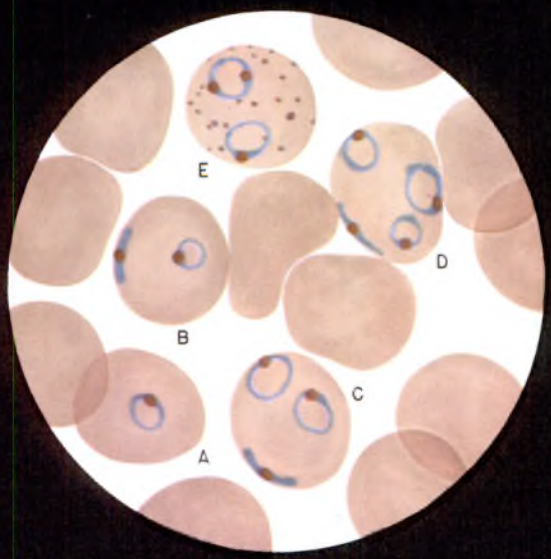
V. Impression Smear of Brain (x 2,000)

- A. Capillary blocked with parasitized erythrocytes. Note the pigment and the deeper staining of the parasites.
- B. Glial cells.
- C. Trophozoite in a free red blood cell.
- D. Maturing schizont.

VI. Section of Liver (x 1,800) - (cells shrunken by fixation)

- A. Küpffer cells containing phagocytized pigment.
- B. Hepatic cells.
- C. Endothelial cell.
- D. Free red blood cells.

PLASMODIUM FALCIPARUM (ESTIVO-AUTUMNAL MALARIA) GIEMSA STAIN



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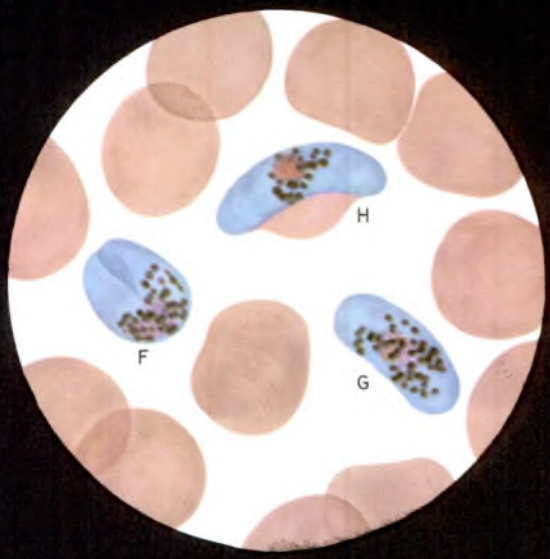

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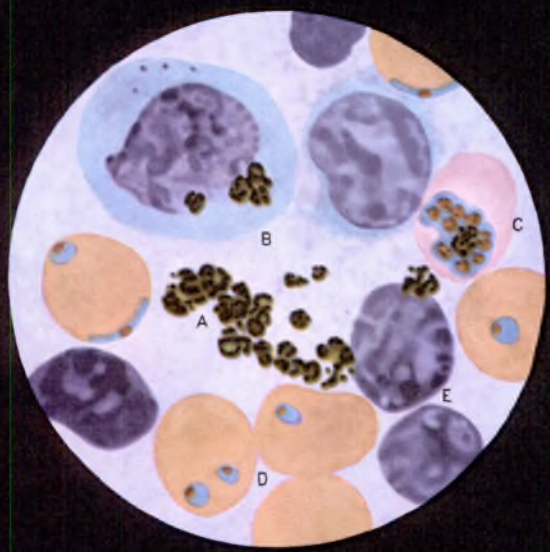

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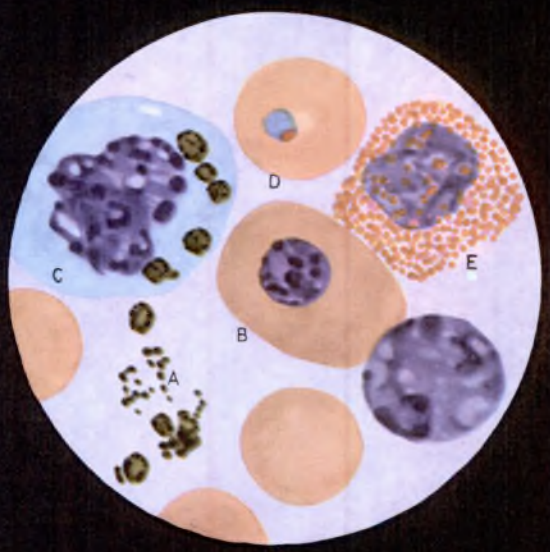

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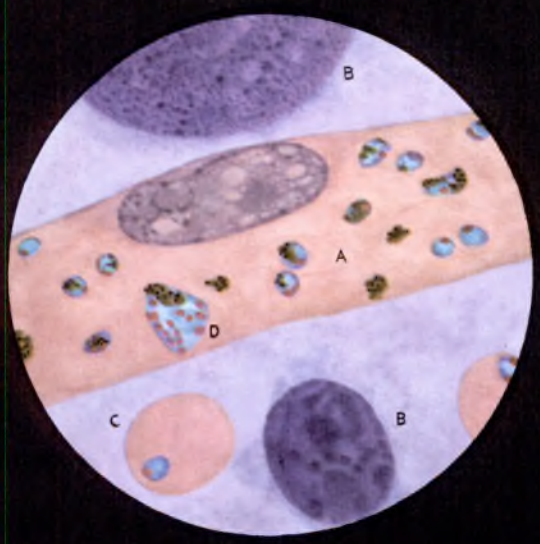

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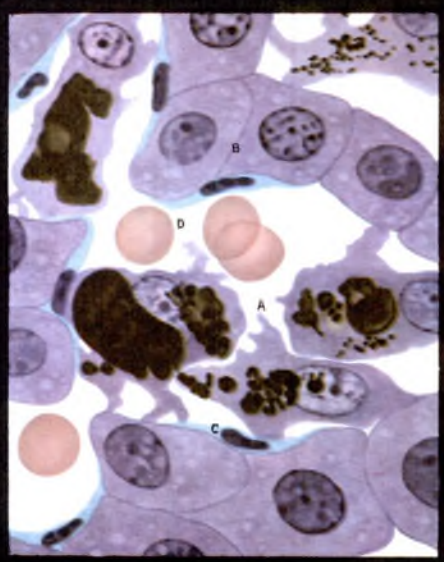


VI

MALARIA PARASITES IN THICK BLOOD FILMS

The thick blood film is a preparation in which a larger quantity of blood than that used in a thin smear is placed on a smaller area of the slide. The hemoglobin of the red cells is removed in the staining procedure, rendering the film transparent. This destroys the red blood cells and distorts the parasites, making species identification more difficult. Four or five minutes is usually ample time for examination with the microscope, and the technic results in more complete study than is possible in 30 minutes with the thin smear. If a thin smear shows 1 parasite per 50 fields, a thick film of the same blood would reveal 5 or 10 parasites in every field. Such a procedure is most valuable in surveys but also has its place in routine clinical work. More positives are found, and the clinical cases are confirmed earlier where this technic is used. The thick film has been estimated to be 25 times better than the thin smear.

Preparation of Film

Blood is obtained from the finger or ear as for blood counting or for the preparation of the thin smear. The skin to be punctured must be clean so that artefacts will not be introduced into the blood specimen. Any alcohol left on the skin may fix the red cells and then they will not clear in the staining procedure. The drop or drops of blood are placed near one end of the slide and spread by stirring with the corner of a fresh slide. Take sufficient blood to give a film about the size of a dime. The blood is allowed to dry in the open air for a minimum of one hour or in the incubator for one-half hour. (Do not overheat to hasten drying, as this also fixes the red cells.) The films are kept flat and protected from dust and insects.

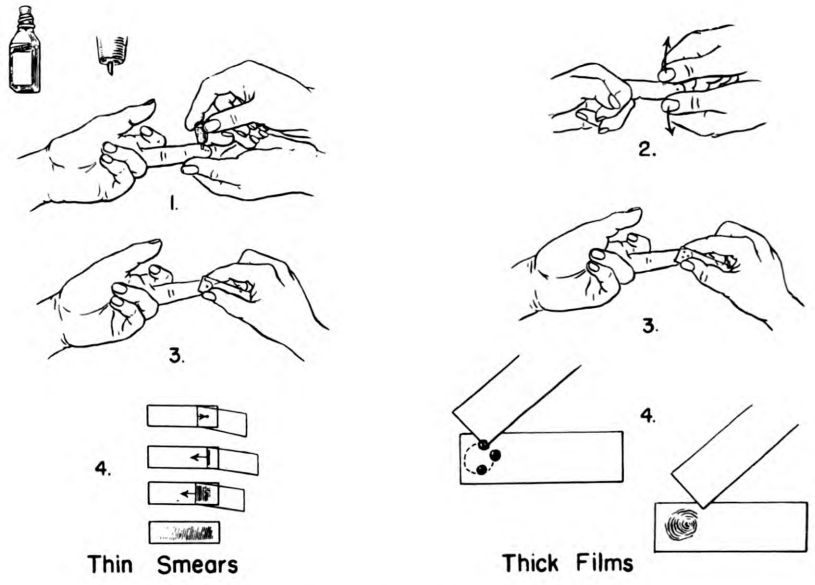


Fig. 23 - Preparation of thick films

Staining

The best stain for this procedure is the Giemsa's preparation available on the supply table. Buffer salts are packaged with the stain so that solutions of known and satisfactory pH will be available.

1. Prepare buffered water by adding about 1.0 gm. (one-fourth teaspoonful) of the buffer salts to a liter of any clean water. Heavily chlorinated water should first be boiled and cooled. This will give a pH which is satisfactory for parasite staining, 6.8 to 7.2.

2. Estimate the amount of diluted stain needed for the slides on hand or for the work of that day. The stock Giemsa will be diluted 1 to 30 or 1 to 50 with buffered water in accordance with directions on the bottle. Measure the required amount of undiluted stain in a small graduate and pour this in the staining dish. Rinse the graduate with some of the measured buffered water, adding this and the remainder of the water to the stain in the dish.

3. Stand the slides on end in this solution. Staining time will vary from 30 minutes to 1 hour or more, depending upon the dilution used and individual experience with that lot of stain. If the films are not dry or are too thick, they may flake off.

4. At the end of the staining time, remove the slides from the stain and stand them on end in a second dish containing buffered water. Thick films should be left in this water from 3 to 5 minutes. Thin smears should be dipped in and removed.

5. Remove the slides from the water and stand them on end upon absorbent paper to dry.

When speed is necessary for diagnostic purposes, thick film preparations as little as 1 hour old may be stained in 5 to 10 minutes in a solution containing 4 ml. of stock Giemsa's stain, 3 ml. of acetone, and 33 ml. of buffered water. This rapid method is not usually used but is of value when speed is essential.

It has been found helpful under some field conditions to lake the thick films for 5 minutes in buffered water before placing them in the stain. Slides that cannot be stained within a short time are best if neither laked nor fixed. They are quite satisfactory after periods as long as 2 weeks and usable after longer periods. The blood film becomes hardened and may stain better if laked as mentioned above.

One milliliter of stock Giemsa is sufficient to stain 25 slides. It should be discarded after staining this number. Stain cannot be safely used the day following its preparation and should be discarded at the end of each day.

Staining Large Numbers of Thick Films

In staining a large number of thick films, it is convenient to handle them in groups of 25 slides. The blood is placed at one end of the slide and the other end is labeled with a wax pencil. The slides are placed in boxes, standing on end so that the slides will be flat if the films are still wet. Pieces of cardboard one-eighth inch thick and one inch square are placed between the slides at the labeled ends. The box is covered with the lid and placed upside down on a flat surface. The bottom of the box is lifted, leaving the slides with the inserted cardboards lying on the lid. The slides are bunched together with the cardboards in place and are secured by several rubber bands placed around the labeled ends. The entire pack is placed in the stain in a vertical position when the films are sufficiently dry. The staining solution should cover the entire film but not reach the cardboards.

Combined Thick Films and Thin Smears

If it is desired to have both thick and thin preparations on the same slide, place the thick film at one end as described. Make a thin film in the center portion of the slide, leaving one end for labeling. Flood the thin smear with methyl alcohol and wash it off with buffered water, being careful that none of the alcohol reaches the thick film. The slide is then immersed in the Giemsa's stain, covering both preparations. The thin smear may be made but not fixed or stained unless it is needed after the thick film has been examined.

Precautions

All slides, cover slips, glassware used for staining, and graduates used for measuring stain must be chemically clean. New slides are preferred, but old slides that have been used only for blood are quite satisfactory. In preparing new slides for use, they should be washed in mild soap solution and hot water, rinsed thoroughly with tap water, and dried after washing with 95% ethyl alcohol. Cleaning solution may cause an acid reaction in the stained preparation. Slides should be protected from dust, and the surface should not be touched with the fingers. All glassware used in mixing the stain or staining should be reserved exclusively for that purpose. Just prior to using, glassware should be rinsed with buffered water.

Pipets should not be introduced into the stock bottle of stain. The stain is poured from the stock bottle into the graduate. The stock bottle must not be left open; where there is high humidity, moisture will reach the stock stain and alter its staining qualities. Direct sunlight has a deleterious effect in time, and reserve bottles should be kept in the dark.

Staining dishes become discolored with use, but this has no significance. At the end of the day they should be rinsed out, using methyl alcohol first, then clean water, and finally buffered water; they are left upside down to drain. The alcohol used for washing dishes can be used repeatedly.

Rapid Method of Staining Thick Blood Films (Field)

The blood films should be about the size of a nickel and not too thick. They are ready to stain as soon as they are no longer obviously moist. Fixation is not necessary. Freshly prepared blood films stain better than when a day or two old.

Solution (A):

Methylene blue	.0.8	gm.
*Azur B (American stains) (German Azure I)	.0.5	gm.
Disodium phosphate (anhydrous)	.5.0	gm.
Potassium phosphate, monobasic (anhydrous)	.6.25	gm.
Distilled water	500	ml.

Solution (B):

Eosin	.1.0	gm.
Disodium phosphate (anhydrous)	.5.0	gm.
Potassium phosphate, monobasic (anhydrous)	.6.25	gm.
Distilled water	500	ml.

The phosphate salts are first dissolved, then the stain is added. Solution of the granular Azur B is aided by grinding in a mortar with a small quantity of the phosphate solution. The solutions of stain should be set aside for 24 hours, and after filtration they are ready for use. The same solutions may be used for many weeks without deterioration, but the eosin solution should be renewed when it becomes greenish from a slight carry-over of methylene blue.

Technic of Staining

1. Dip the film for 1 to 5 seconds into solution (A).

2. Remove from solution (A) and immediately rinse by waving gently in clean water for a few seconds until the stain ceases to flow from the film and the glass of the slide is free from stain.

3. Dip for 1 to 5 seconds into solution (B).

4. Rinse by waving gently for 2 or 3 seconds in clean water.

5. Place vertically against a rack to drain and dry.

The concentration of the stain is adjusted for staining times of 1 second with an immediate wash of 5 seconds, but relative times may need slight adjustment to suit different batches of stain.

*Should Azur B be unobtainable, it is possible to prepare a methylene blue-azure mixture of undefined composition from the medicinal methylene blue. Solution (A) may be prepared as follows:

PLASMODIUM VIVAX (Thick smears stained by Giemsa, pH 6.8) (x 1,000)
(Plate 9)

I. Polymorphonuclear neutrophile leukocyte at 12 o'clock.
Many young trophozoites ("ring forms") scattered over the field (these are not diagnostic for species).
One mature schizont just to right of center.

II. Polymorphonuclear neutrophile leukocyte at 9 o'clock.
Four slightly later trophozoites, showing beginning amoeboid activity, scattered over the field.
One microgametocyte (male gametocyte) at 2 o'clock. Note diffuse chromatin in nucleus and pale blue cytoplasm.

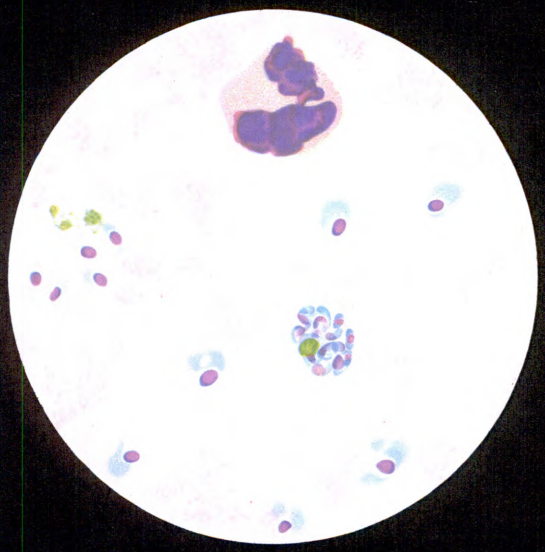
III. Polymorphonuclear leukocyte at 5 o'clock.
Six mature trophozoites scattered over the field. Note the amoeboid activity and pigmentation.

IV. Eosinophilic leukocyte at 1 o'clock.
Two early schizonts at about a 4-nucleus stage at 7 and 10 o'clock.
Remains of a macrophage with phagocytosed blood pigment just off center at 3 o'clock.

V. Polymorphonuclear neutrophile leukocyte at 3 o'clock.
Two mature schizonts not far off center at 4 and 11 o'clock.
Macrogametocyte (female gametocyte) at 8 o'clock. Note concentrated mass of chromatin in nucleus and bright blue cytoplasm.

VI. Polymorphonuclear neutrophile leukocyte at 11 o'clock.
Early schizont, 6-nucleus stage at 3 o'clock.
Remainder of parasites on field are young amoeboid trophozoites.
All parasites in this field are surrounded by the remnants of the stroma of erythrocytes.

PLASMODIUM VIVAX (Thick smears stained by Giemsa, pH 6.8)



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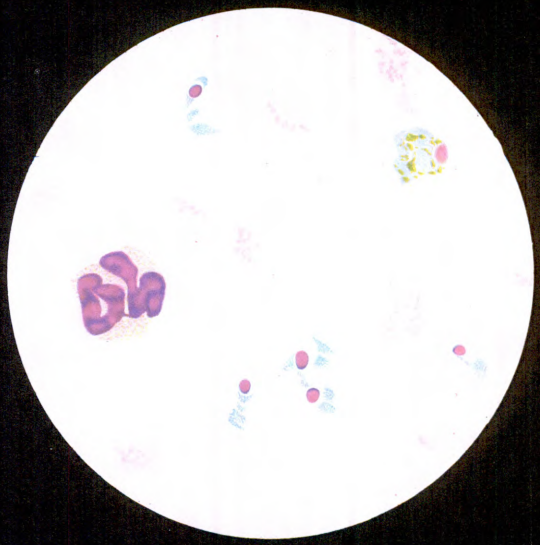

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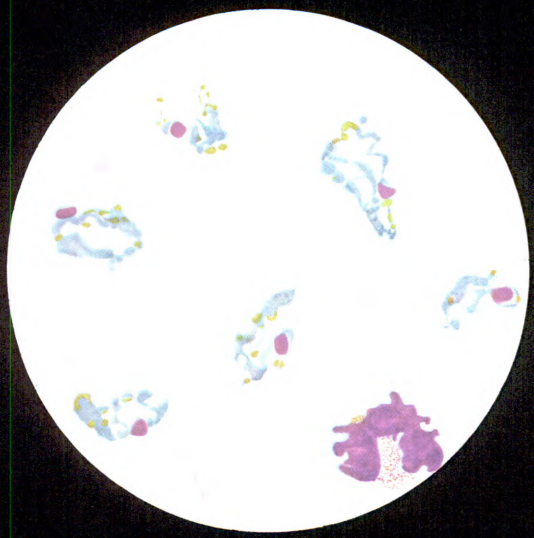

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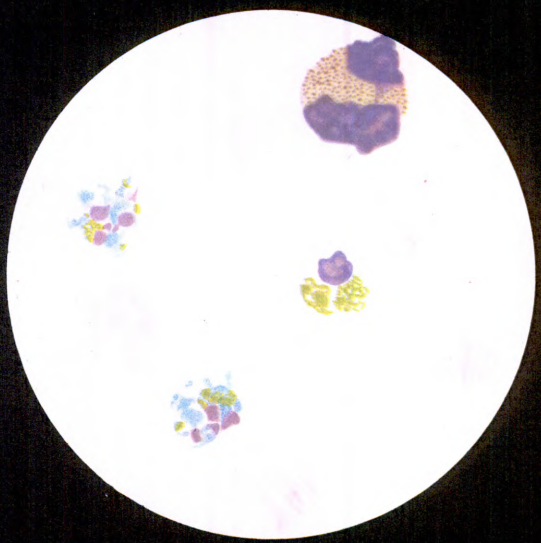

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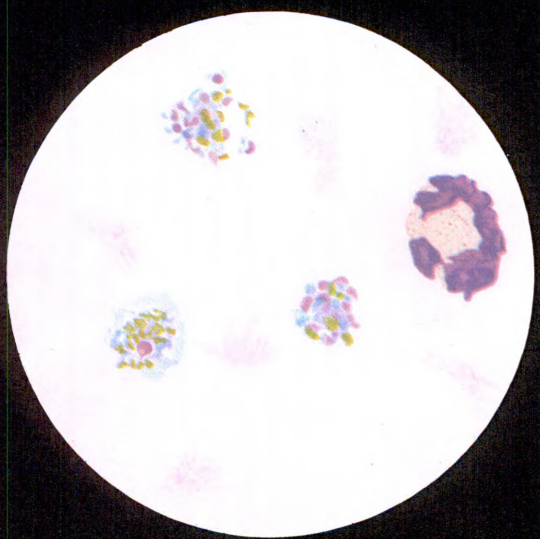

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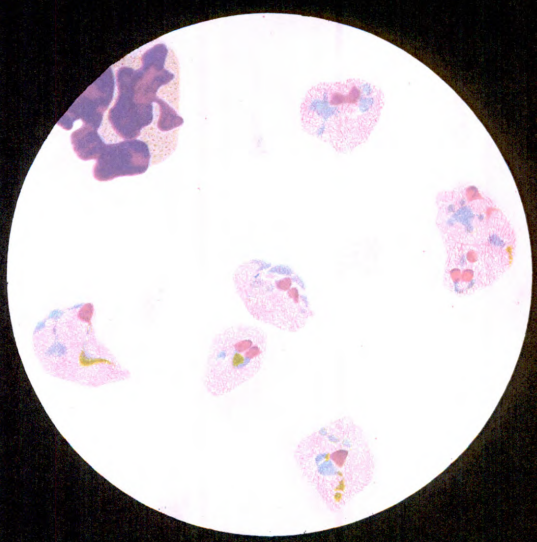


VI

NAVAL MEDICAL SCHOOL '48

1. Dissolve 1.3 gm. of medicinal methylene blue and 5.0 gm. of anhydrous disodium phosphate in 50 ml. of distilled water.

2. Bring to the boil and then evaporate in a water bath almost to dryness.

3. Add 6.25 gm. of anhydrous potassium phosphate, monobasic.

4. Add 500 ml. of distilled water, stir until the stain is completely dissolved, and set aside for 24 hours.

5. Filter before use.

DETECTION AND IDENTIFICATION OF MALARIA PARASITES IN THICK BLOOD SMEARS

The detection of parasites in the thick films depends first upon the recognition of colors. This requires a bright light, the intensity depending upon individual choice. A blue filter may be found objectionable to the beginner as it may obscure the red of some of the parasites. The focus of the microscope must be constantly altered since the thick film has an appreciable depth.

In general, the parasites have the same characteristics that identified them in the thin smears. Some points of identification have been removed in destroying the red blood cells and the parasites are modified. Shadows or skeletons of red blood cells and Schüffner's granules may be seen in P. vivax infections. When difficulties in identification are encountered, the margin of the thick film should be searched; here the parasites more nearly resemble their appearance in the thin smear. All thick preparations will show distorted nuclei of leucocytes, platelets, and debris. Platelets are frequently mistaken for parasites. Various rounded objects may take a red tint from the stain; to add to the confusion, they may be on or near a mass of blue debris.

The two principal structures of the parasite, the red nucleus or nuclei and the blue cytoplasm, must be seen to identify a parasite. In the thick film, the nucleus or chromatin dot stands out prominently and appears more compact than in the thin smear; it stains pink, scarlet, or red. The cytoplasm varies in shape and shade of blue according to species and form. When the red cell is destroyed by the stain, the parasites may become distorted into bizarre shapes. As the rings of young trophozoites collapse, the cytoplasm forms a tail of blue which may or may not be visibly attached to the chromatin. The delicate cytoplasm of P. falciparum may be barely visible and often appears to be less in amount than the chromatin. The pigment is practically the same as in the thin smears. White cells containing pigment, considered to have diagnostic importance, are rarely seen.

The thin smear is to be preferred if the species diagnosis must be made from the examination of a very few parasites. However, when the thin smear shows a

few parasites, the thick film will show many. Diagnostic forms will usually be found when many parasites can be examined. Young trophozoites are no more diagnostic than they were in the thin smears. P. vivax and P. malariae rarely, if ever, show only ring forms, so the observation of many parasites becomes important. Nearly all clinical cases will show parasites in the thick film, while some thin smears would be called negative.

Again the differential diagnosis of species must be made from the asexual forms. Gametocytes are more confusing than helpful. Crescents may be seen on end or folded over and may be mistaken for trophozoites or schizonts of P. malariae. Macrogametocytes of either P. vivax or P. malariae cannot be differentiated from mature trophozoites. The microgametocytes of these two species are distinctive in their relatively large nuclei, more deeply stained than in the thin smear, surrounded by light staining or colorless cytoplasm containing characteristic pigment granules.

Morphology

Forms present:

P. vivax	All stages of development
P. malariae	All stages of development
P. falciparum	Only rings or rings and crescents

Young trophozoites (least diagnostic) - As the ring form collapses, the cytoplasm becomes variously arranged in relation to the nucleus. Rarely the ring form will persist; sometimes the cytoplasm appears as a broken ring; usually it appears as a blob or dash of blue; it may be on one or both sides of the chromatin. Various descriptive terms have been applied: "swallow" form when there is a dash of blue on each side of the chromatin; "comma" form when the cytoplasm becomes a curved thread; "flagstaff" form when there is a dash of blue extending from the red dot.

P. vivax	Larger than others with heavier chromatin dot; cytoplasm in strings, farther from chromatin
P. malariae	The medium-sized species; chromatin dot may be as large as P. vivax; cytoplasm close to chromatin
P. falciparum	Small and delicate with tiny chromatin dot; double chromatin dots; not sufficient cytoplasm to extend far from the chromatin

PLASMODIUM MALARIAE (Thick smears stained by Giemsa, pH 6.8) (x 1,000)
(Plate 10)

I. Polymorphonuclear leukocyte at 12 o'clock.
Young ring forms at 5 o'clock and 9 o'clock (not diagnostic for species).
Mature schizont, six-nucleus stage, at 2 o'clock.
Mature schizont, eight-nucleus stage, just off center of field. Note large amount of pigment and "daisy-head" configuration.

II. Polymorphonuclear leukocyte at 11 o'clock.
Microgametocyte (male gametocyte) at 7 o'clock. Note that chromatin is centrally placed, pale blue staining; cytoplasm is pale, almost colorless; and large amount of pigment is present.
Remainder of structures on field are mature trophozoites. Note compactness of cytoplasm and large amount of pigment.

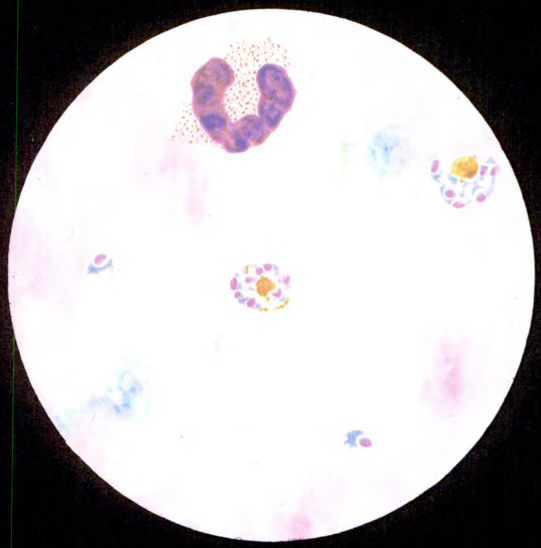
III. Polymorphonuclear leukocyte at 11 o'clock.
Macrogametocyte (female gametocyte) at 7 o'clock. Note compact chromatin and blue staining of cytoplasm.
Five-nucleated schizont at 2 o'clock. Note large amount of pigment.
Two-nucleated schizont at 5 o'clock.

PLASMODIUM FALCIPARUM (Thick smears stained by Giemsa, pH 6.8) (x 1,000)
(Plate 10)

IV. Polymorphonuclear leukocyte at 11 o'clock.
Remainder of structures on field are young trophozoites ("ring forms"). These are not diagnostic for species.

V. Polymorphonuclear leukocyte at 10 o'clock.
Crescent-shaped gametocyte at 9 o'clock, not far out from center. This form of gametocyte is characteristic of this species.
Two distorted gametocytes, probably microgametocytes, at 1 o'clock and 4 o'clock.

PLASMODIUM MALARIAE (Thick smears stained by Giemsa, pH 6.8)



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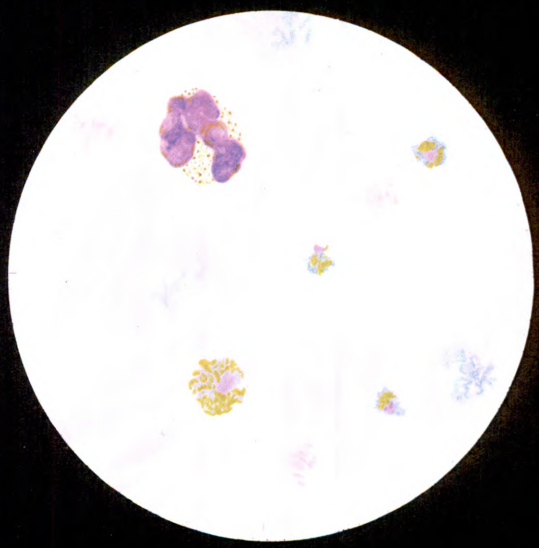

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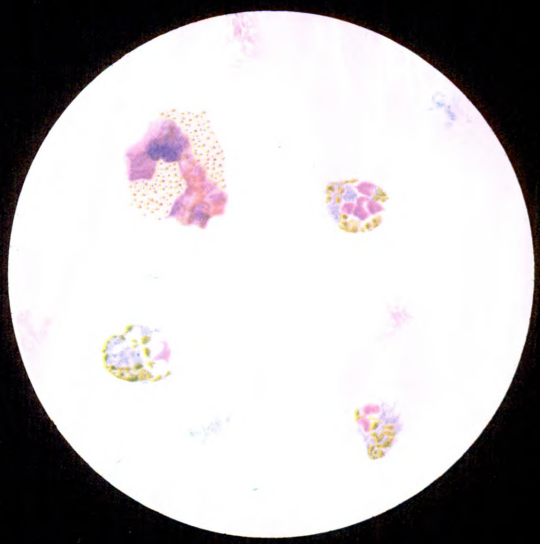
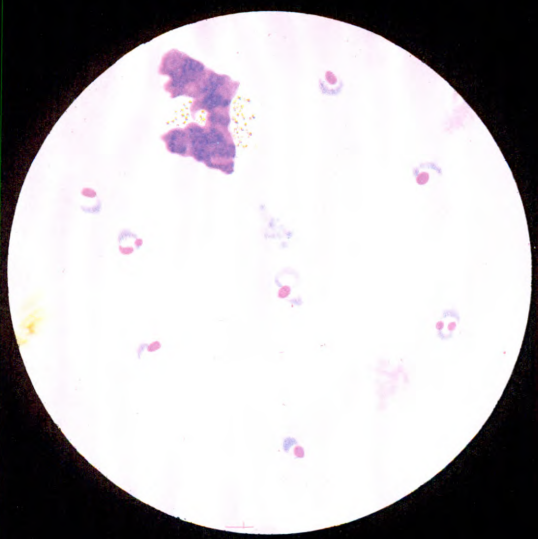


III

PLASMODIUM FALCIPARUM (Thick smears stained by Giemsa, pH 6.8)



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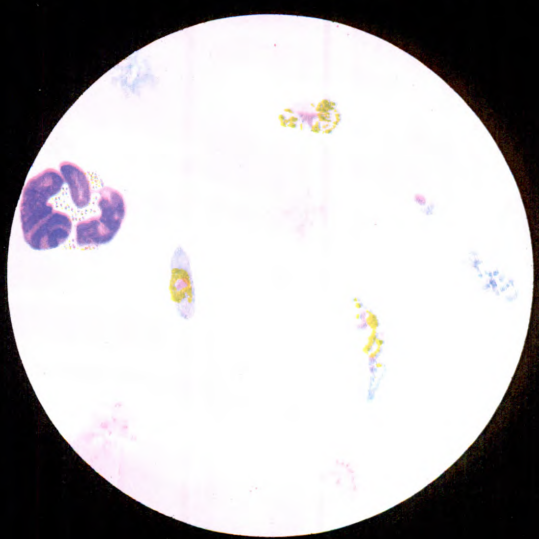


V

NAVAL MEDICAL SCHOOL '48

Growing and large trophozoites:

P. vivax	Abundant masses and blobs of light blue cytoplasm connected by threads or not visibly connected; arranged in Y-forms or triangles; ameboid activity reflected. Older forms with fine yellow-brown pigment, irregular; chromatin round or irregular
P. malariae	Single blob of deep-staining cytoplasm, compact and close to nucleus; usually rounded, no band forms. Older forms, chromatin may be obscured by crowding cytoplasm or elongated; heavily pigmented, dark-brown to black coarse granules
P. falciparum	Very much like young P. vivax, possibly more compact; may still show double dot forms; occasionally pigmented

Schizonts:

P. vivax	Chromatin masses irregular in contour in immature forms; 12 or more nuclei in older forms; pigment clumped
P. malariae	Pigment more abundant; 12 or less nuclei in older forms, characteristic arrangement in "rosette" or "daisy" pattern
P. falciparum	Rarely seen; small in size of parasite and size of nuclei; pigment dark to black

POSTMORTEM DEMONSTRATION

It is sometimes necessary to make postmortem examinations in order to find the parasites of P. falciparum. In rare cases of death from P. vivax or quartan infections, parasites may be recovered from the peripheral blood or heart's blood. When death from P. falciparum infection is suspected — in all areas where this species is endemic or where the deceased has lived in such an endemic area within the last 6 months — the following procedure should be carried out:

Smears or impressions should be taken of the spleen, bone marrow, liver, and gray matter of the brain. To do this a clean slide is pressed tightly against the cut surface of spleen or liver, and the pulp is made to extrude by light pressure on the pieces of tissue held between the fingers. Bone marrow impressions are easily

obtained by pressing a sectioned rib between the blades of bone forceps. Smears from the brain are obtained by crushing a small bit of the gray matter of the cortex between two slides and dragging the slides apart as in the making of a thin blood smear. When the smears and thin impressions have been made, they should be fixed in methyl alcohol and stained with Giemsa's, or they may be stained with Wright's, using exactly the same method as for a thin blood film.

In the fixation of blocks of tissue for section, formalin fixatives should be avoided. Formalin produces an abundant precipitate which may be easily mistaken for true malarial pigment, and the parasite, after formalin fixation, does not take the stain so well as after Zenker's fixative. Zenker fixation is used for most tissues infected with protozoa.

Zenker's fixing fluid:

Potassium bichromate	2.5	gm.
Bichloride of mercury (corrosive sublimate)	5	gm.
Water	100	ml.
Glacial acetic acid	5	ml.

Dissolve bichromate and sublimate in water with the aid of heat. This is stock solution. Keep acetic acid in a separate bottle until the fixing fluid is to be used. It will produce changes in chrome salt if added at once and allowed to stand.

Caution - The tissue must not remain in Zenker's more than 24 hours. On removal it should be washed in running water 24 hours and then preserved in 70% alcohol tinted yellow with iodine.

The following method is suggested by Lendrum (Brit. Med. Jour., 8 July 1944) for tissues: Fix in an aqueous solution of 2.5% potassium bichromate and 5% mercuric chloride for about 6 hours; then transfer the tissues directly to aqueous 5% mercuric chloride; fixation continues in this without danger of spoiling the common methods of staining, as may result from the prolonged action of bichromate.

BLOOD AND TISSUE FLAGELLATES

CLASSIFICATION

Phylum: Protozoa
Class: Mastigophora
Order: Protomonadidae
Family: Trypanosomidae
Genus: Trypanosoma
Species in man: gambiense, rhodesiense, cruzi

Genus: Leishmania
Species in man: donovani, tropica, brasiliensis

The Family Trypanosomidae consists of simple flagellates which differ widely in their parasitism. Some are parasites of plants, many live in the alimentary tracts of insects and are passed from insect to insect, and a number infect vertebrates but utilize invertebrates as intermediate hosts. In addition to those listed above, several species are found in other vertebrates; a few of these are:

Species	Definitive Hosts	Vector	Distribution	Disease
T. brucei	Domestic animals, Wild game	Tsetse flies	African	Nagana
T. lewisi	Rats	Fleas	Cosmopolitan	None
T. equiperdum	Horses, Ass	None (by coitus)	Cosmopolitan	Dourine
T. evansi	Domestic animals	Horseflies	Cosmopolitan	Surra

MORPHOLOGY

The blood and tissue flagellates go through morphological changes during their life cycle, all having at some time an elongated body, a single nucleus, and a single flagellum. In some species these changes take place in the vertebrate host, and in others they are apparent only in the arthropod host or upon culture. Some forms are within the cells, others are extracellular. The stages commonly recognized are illustrated in the accompanying figure. Multiplication is by longitudinal fission.

Leishmanian Form

This is the usual intracellular stage. The body is round or ovoid and has no flagellum or undulating membrane.

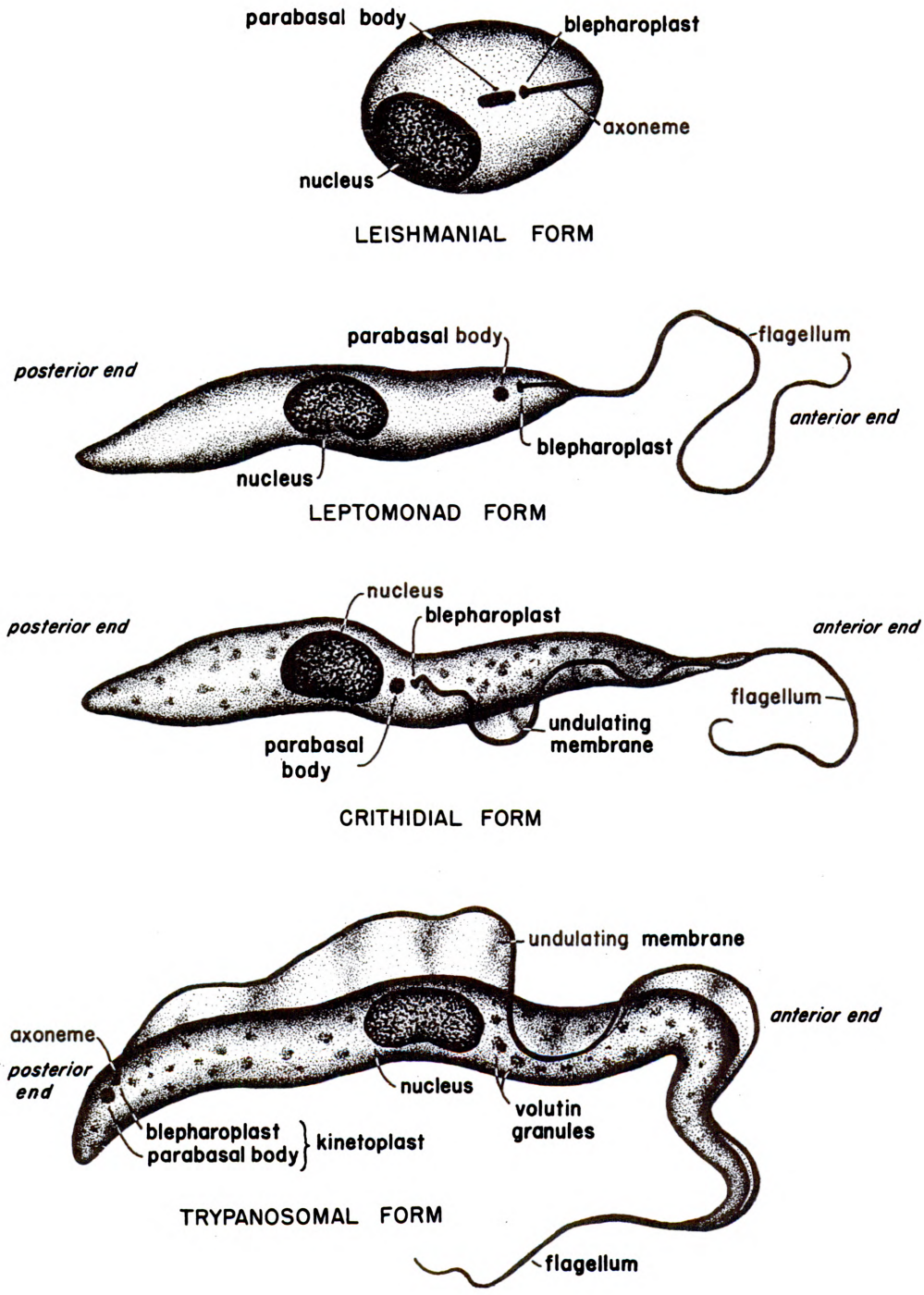


Fig. 24 - Morphological forms of the hemoflagellates

Leptomonad Form

The body is elongated and somewhat flattened. The flagellum arises from the anterior tip of the body and there is no undulating membrane.

Crithidial Form

Both undulating membrane and flagellum are present. The kinetoplast, the term assigned to the blepharoplast and the parabasal body, is just anterior to the nucleus.

Trypanosomal Form

This is the form usually found in blood smears. It differs from the crithidia only in that the kinetoplast is posterior to the nucleus.

Metacyclic Trypanosome

This is the infective form found in the insect host. These organisms are present in the salivary secretions in some species in which case they are called anterior station parasites. In other species they are found in the feces, being called posterior station parasites.

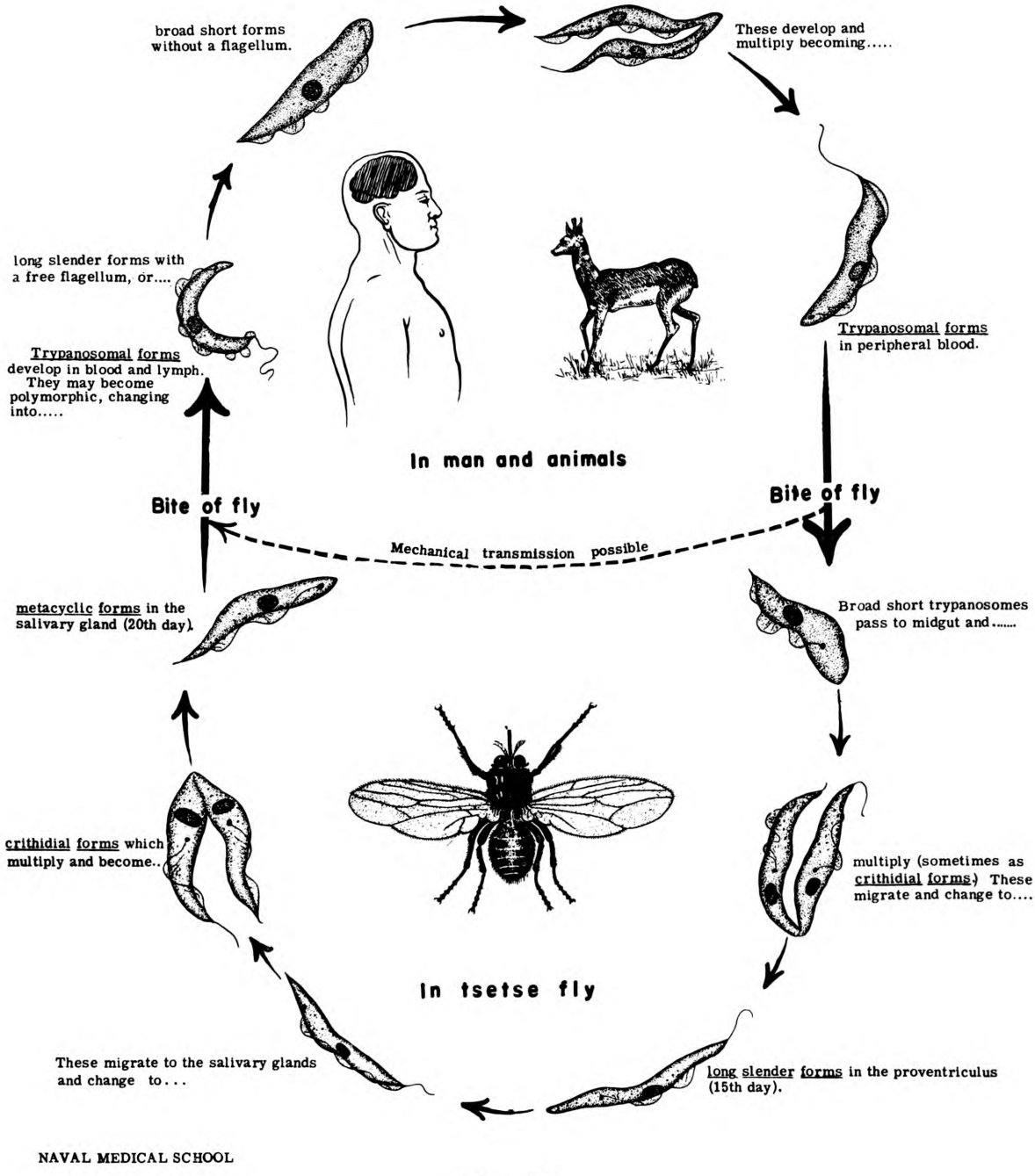
TRYPANOSOMA GAMBIENSE AND TRYPANOSOMA RHODESIENSE

These parasites produce a disease of man commonly called African sleeping sickness or trypanosomiasis. Since the two species are very similar, they are considered together. Trypanosoma gambiense is found in West Africa and T. rhodesiense in East Africa.

Morphology

The unstained trypanosome appears as a colorless, slender body, 15 to 30 microns long. It is actively motile in fresh blood, but it makes little headway; the undulating membrane and flagellum may be visible. Stained with Wright's or Giemsa's stain, the nucleus is red and the cytoplasm pale blue; the kinetoplast stains purple. The relative position of these two bodies has been noted (Fig. 24). The flagellum appears as a fine red thread along the edge of the undulating membrane, free at the anterior end. These trypanosomes are polymorphic, some showing altered shapes and having no free flagellum. When Trypanosoma rhodesiense is inoculated into rats or guinea pigs, posterior nucleate forms develop, the nucleus being located near the posterior end. The two species are indistinguishable as seen in human blood.

LIFE CYCLE OF TRYPANOSOMA GAMBIENSE AND TRYPANOSOMA RHODESIENSE



NAVAL MEDICAL SCHOOL

Fig. 25

Life Cycles

Since the life cycles of both species are identical, only one is presented in the accompanying figure. There are two morphological types; leptomonad and leishmanian forms do not occur. The parasite is transmitted by flies of the genus Glossina. Development in the fly requires about 20 days. Domestic and wild animals serve as a reservoir of infection.

Laboratory Diagnosis

The trypanosomes may be recovered from lymph glands, blood, or spinal fluid. Early in the disease, best results are obtained by examination of material obtained from an enlarged lymph gland. Later the parasites may be readily recovered from the blood and occasionally from the spinal fluid. Cultures and animal inoculation may be employed when direct examination fails to confirm the diagnosis. However, cultures of Trypanosoma gambiense and T. rhodesiense are less satisfactory than with the other haemoflagellates.

1. Fresh blood smears - A very small drop of blood is placed on the glass slide and spread by placing a cover slip on it. Movement of the parasites causes a characteristic movement of the surrounding blood cells. The examination will fail if too much blood is used.

2. Stained blood smears - Thick or thin smears, stained as for malaria parasites, are satisfactory for trypanosomes.

3. Blood concentration - Blood is collected in 2% citrate — physiological sodium chloride solution. After filtering through cheesecloth, it is centrifuged for 20 minutes. The cells are separated and hemolyzed by adding 2% acetic acid or distilled water. After centrifuging again for 30 minutes, smears are made of the sediment and stained for parasites.

4. Aspiration - A small amount of saline may facilitate the aspiration of lymph glands for trypanosomes. The lymph juice fluid should be examined for motile organisms, stained, and cultured. Spinal fluid should be centrifuged and the sediment given similar treatment.

5. Animal inoculation - Inject 2 to 10 ml. of blood into a guinea pig, mouse, rat, cat, or dog; they may not become positive for a week or more.

6. Culture - This is difficult. Brutsaert and Henrard's (1936)* medium is probably the best.

TRYPANOSOMA CRUZI

This flagellate is transmitted by various species of reduviid bugs and causes Chagas' disease (American trypanosomiasis) found in Central and South America.

*Brutsaert, P. et Henrard, 1936. La cultures des trypanosomes pathogénes; Ann. Soc. Belge Med Trop; 16:479-481. (Review by J. Rodhain)

Morphology

Leishmanial and trypanosomal forms (Fig. 24) occur in man or other mammalian hosts while crithidial and trypanosomal stages may be found in the insect hosts. Extracellular trypanosomes and the intracellular leishmanian forms are more commonly seen. The unstained trypanosome in the blood smear resembles Trypanosoma gambiense; it is slightly smaller, averaging 20 microns. It is frequently curved into C- and U-shapes when stained. The body is short and broad with a pointed posterior end. Dividing trypanosomal forms are not present. The leishmanian forms are usually demonstrated in the heart and other tissues. They are 2 to 4 microns in diameter, usually present in colonies or groups within the cells or between the muscle fibers. In the stained preparations they show a red nucleus and a purple rod-like kinetoplast.

Life Cycle

The parasite recognized in the blood as the trypanosomal form changes to the leishmanian form found in the various tissues. The latter multiply by binary fission and probably develop leptomonad and crithidial forms which are liberated by rupture of the cells. Crithidial forms develop in the insect vector, multiply, and become metacyclic forms which are passed in the feces. Man is infected when an abrasion or the wound produced by the insect's bite is contaminated with feces. Numerous animals serve as a reservoir of infection.

Laboratory Diagnosis

1. Blood may be examined as for Trypanosoma gambiense but is rarely positive except during febrile periods.

2. Spinal fluid has rarely shown Trypanosoma cruzi.

3. Animal inoculation - Inject 5 to 10 ml. of blood into guinea pigs or puppies. Their blood may be positive after 2 weeks.

4. Xenodiagnosis - Reduviid bugs are allowed to feed on the patient. Any ingested trypanosomes will rapidly multiply and can be recovered in about 2 weeks. Since insects may be naturally infected, only laboratory reared bugs are used.

5. Culture - The N.N.N. medium is recommended, prepared as follows: agar, 14 gm.; sodium chloride, 6 gm.; distilled water, 900 ml. Mix, bring to a boil, place in tubes, and sterilize. In using, melt the agar tube, cool to 48° C., and add one-third its volume of defibrinated rabbit's blood. Slant the tube and cool on ice to produce the maximum water of condensation in which development occurs best. Culture at room temperature. Senekjie's medium (1939)* may give better results.

6. Postmortem - Since a common location is the heart, leishmanian forms may be found in sections of this organ fixed in Zenker's solution.

*Senekji, H.A., 1939. Studies on the culture of Leishmania tropica. Trans. Roy. Soc. Trop. Med. and Hyg. 33:267-299; or see Faust, E.C., 1955. Animal Agents and Vectors of Human Disease, P. 605.

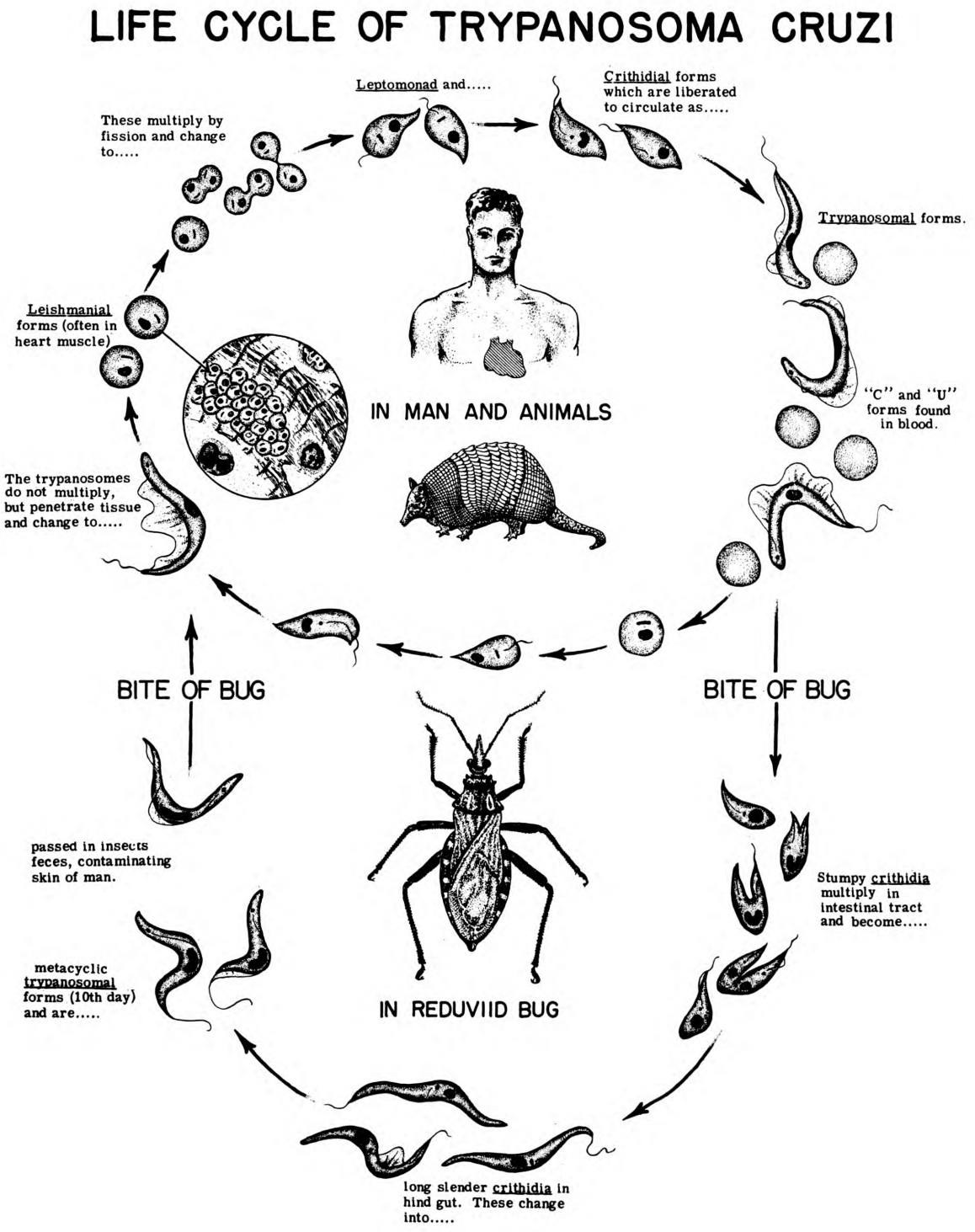

LIFE CYCLE OF TRYPANOSOMA CRUZI
Leptomonad and.....
Crithidial forms which are liberated to circulate as.....
These multiply by fission and change to.....
Trypanosomal forms.
Leishmanial forms (often in heart muscle)
"C" and "U" forms found in blood.
IN MAN AND ANIMALS
The trypanosomes do not multiply, but penetrate tissue and change to.....
BITE OF BUG
BITE OF BUG
passed in insects feces, contaminating skin of man.
Stumpy crithidia multiply in intestinal tract and become.....
metacyclic trypanosomal forms (10th day) and are.....
IN REDUVIID BUG
long slender crithidia in hind gut. These change into.....

Fig. 26

7. Complement fixation - Cultures of Trypanosoma cruzi are used as antigen with good results.

LEISHMANIA DONOVANI

The disease known as kala-azar or visceral leishmaniasis is caused by this parasite. It is more widely distributed than the other Leishmania, being found in the Mediterranean countries, Asia Minor, southern Russia, India, Turkestan, northern China, Manchuria, tropical Africa, Argentina, and Brazil.

Morphology

Leishmania donovani exists in the tissues of man only in the leishmanian form - intracellular, nonflagellated, oval bodies. They may be present in small numbers in the blood but are found in nearly all the internal organs, especially in the reticulo-endothelial cells. The structure of this form can be seen only when stained. With Wright's or Giemsa's stain, a large red nucleus and a purple rod-shaped parabasal body are visualized. A single macrophage may show many of these leishmanian bodies.

Life Cycle

The leishmanian form in man or animals is ingested by sand flies of the genus Phlebotomus. Leptomonad forms develop in the insect as well as upon culture. The natural host appears to be the dog, although other animals seem to harbor the parasites.

Laboratory Diagnosis

1. Blood smears are most available and first examined. Both thin and thick preparations may be made. Since the technic used for malaria will destroy the parasites in the thick film, special laking is required. Flood the dried film with an aqueous solution of 2.5% glacial acetic acid, 4 parts, and 2% crystalline tartaric acid, 1 part, for 5 to 10 minutes. Drain off the solution, fix with methyl alcohol, wash thoroughly, and stain with dilute Giemsa's. Search for the parasites within the leucocytes.

2. Concentration - Add citrated Locke's solution to the blood and centrifuge at 750 revolutions for 5 minutes. Examine the sediment by stained smear or culture.

3. Aspiration - Spleen, liver, gland, or bone marrow puncture may produce material which will disclose the parasites. Splenic puncture will reveal a higher percentage of positives than liver puncture, but both procedures are apparently being replaced by the sternal puncture which is safer and more simple. The material withdrawn by aspiration resembles thick blood. It may be smeared directly on the

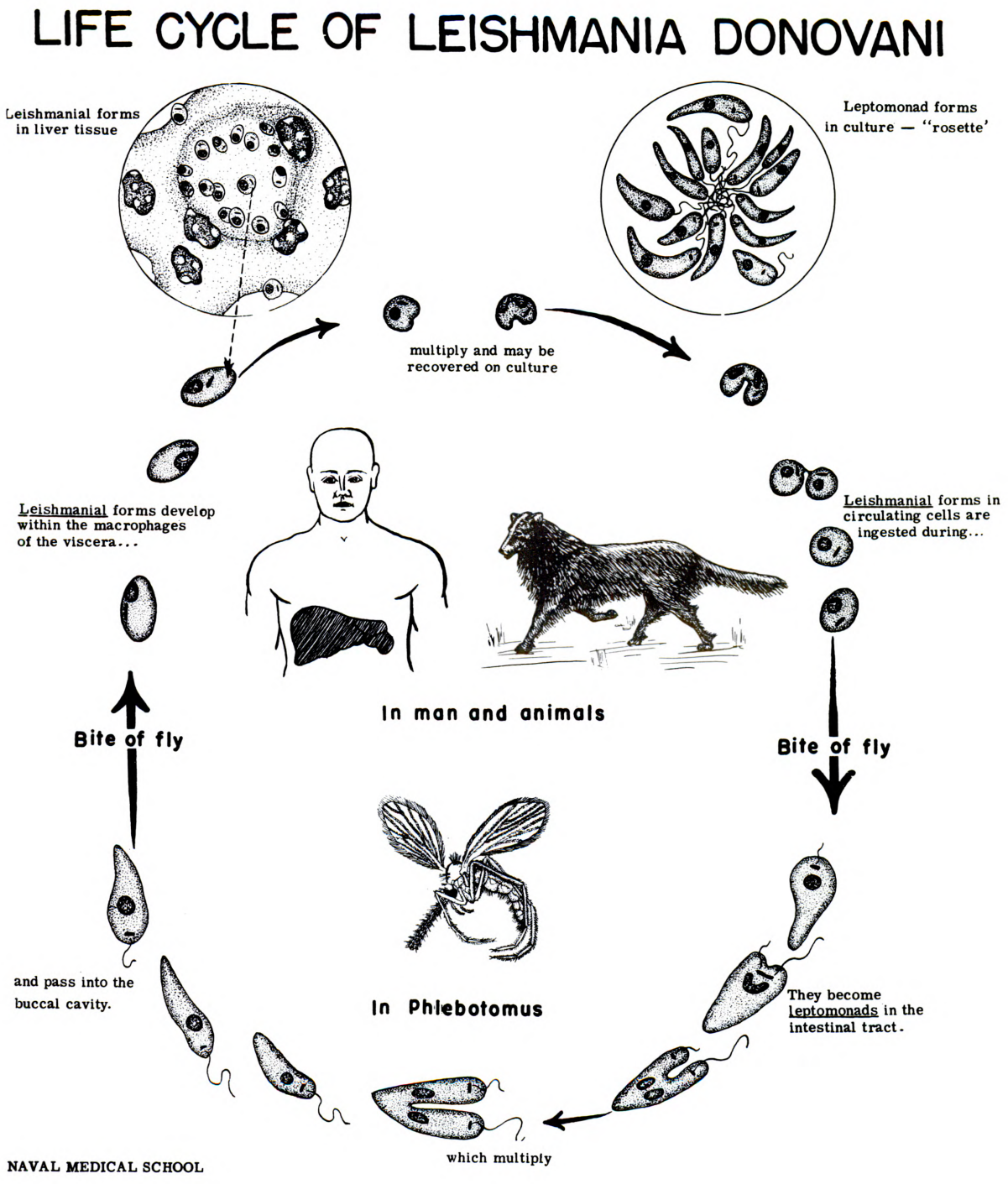

LIFE CYCLE OF LEISHMANIA DONOVANI
Leishmanial forms in liver tissue
Leptomonad forms in culture — "rosette'
multiply and may be recovered on culture
Leishmanial forms develop within the macrophages of the viscera...
Leishmanial forms in circulating cells are ingested during...
In man and animals
Bite of fly
Bite of fly
In Phlebotomus
and pass into the buccal cavity.
They become leptomonads in the intestinal tract.
which multiply
NAVAL MEDICAL SCHOOL

Fig. 27

slides for staining or oxalated in a test tube for later examination. Needles and syringes must be dry since water destroys the leishmania.

4. Culture - Senekjie's (1939) and the N.N.N. medium are recommended. Usually there is no satisfactory growth before the seventh day, and it is well to leave the culture untouched until the tenth day. Leptomonads similar to those found in insects develop in the culture.

5. Serological and biochemical tests:

a. Aldehyde test (Napier) - Add one drop of 40% formaldehyde to 1.0 ml. of patient's blood serum in a test tube, shake well, and allow to stand at room temperature. If positive, the serum immediately becomes opaque and gels within 3 to 30 minutes. If negative, no reaction should occur in 24 hours. Infection of less than 4 months' duration may produce an opaque serum which does not gel; such doubtful reactions should be confirmed by other tests. A finger-prick test is also available.

b. Antimony test (Chopra) - Place 0.2 ml. of whole serum and an equal amount diluted 1:10 with distilled water in two small tubes. Carefully overlay with a 4% solution of pentavalent antimony (ureastibamine). If positive, a thick flocculent disc forms at the junction of the two fluids. Reactions which are delayed for a few minutes to an hour suggest an early infection.

c. Precipitation test (Sia) - This precipitation test is suggestive evidence of kala-azar. Place 20 cu. ml. of blood in a small test tube containing 0.6 ml. of distilled water and agitate until mixed. An immediate clouding of the distilled water indicates a positive test. The time required for sedimentation — 15 minutes to 1 hour — shows the strength of the reaction.

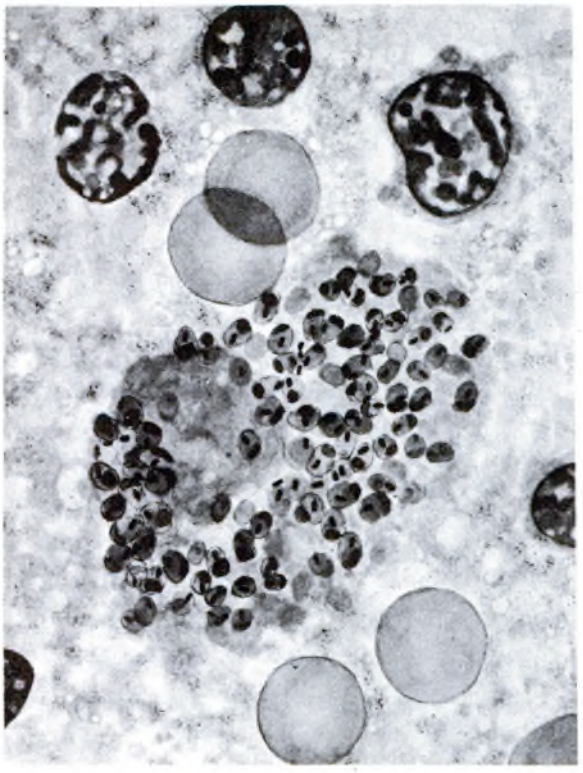
d. Complement fixation - A test is available but is only moderately successful.

LEISHMANIA TROPICA

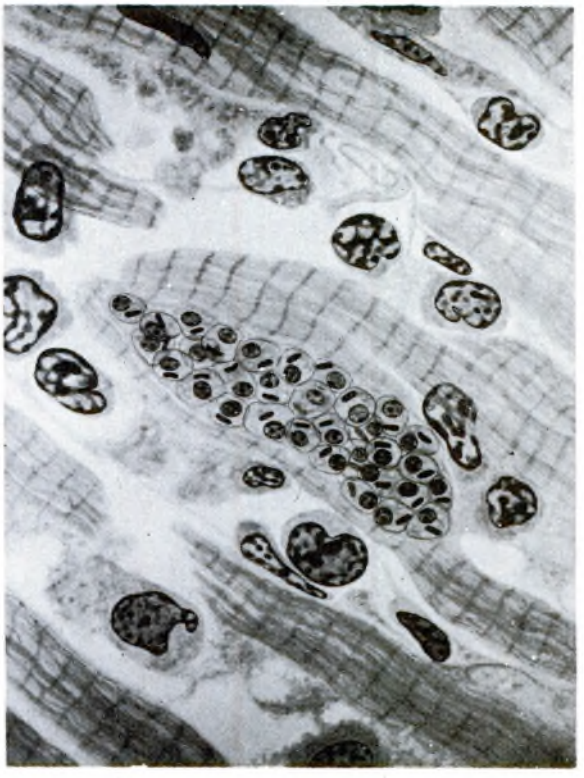
Leishmania tropica is the causative agent of cutaneous leishmaniasis or oriental sore. It is found in many of the countries having kala-azar but not in the same localities. It is usually considered a disease of the Near East and Mediterranean area.

Morphology

The forms are indistinguishable from those of Leishmania donovani; they have been differentiated by serological methods.



L. donovani in smear of spleen



T. cruzi in section of heart muscle

T. lewisi in blood smear from rat

Crithidial form Leptomonad form

From culture of L. donovani

Fig. 28

Life Cycle

The life cycle is the same as for Leishmania donovani, except that the parasite invades the reticulo-endothelial cells of the skin rather than those of the viscera in man. The insect vector is the same, and comparable lesions are found on dogs where this disease occurs.

Laboratory Diagnosis

Material for examination should be obtained from the cutaneous lesions rather than from blood or viscera. The skin lesion may be scraped or serum aspirated from the surrounding induration. Stained smears or cultures may be used as for Leishmania donovani.

LEISHMANIA BRASILIENSIS

This parasite causes American leishmaniasis or mucocutaneous leishmaniasis; also called espundia, uta, and forest yaws. The disease is almost exclusively confined to Central and South America. Recently a case has been reported from Texas.

Morphology

The forms are indistinguishable from Leishmania donovani and L. tropica.

Life Cycle

The life cycle is incompletely known. The skin and mucous membrane of the nose, mouth, and pharynx are principally involved. The vector is believed to be the same as for Leishmania tropica and L. donovani.

Laboratory Diagnosis

Procedures as outlined for Leishmania tropica are recommended.

TISSUE AND BLOOD PARASITES OF UNCERTAIN RELATIONSHIP

Toxoplasma gondii

Only recently has the possible importance of infection by this parasite been recognized. Toxoplasma occurs in a large number of birds and mammals including man. Although cosmopolitan in distribution there is only sketchy information on the epidemiology of infections in man or other animals. Toxoplasma is primarily a parasite of the reticulo-endothelial system. It has been found in the eyes, the

brain and lungs as well as in wandering macrophages of the circulatory system or in exudate from the peritoneal, pleural and cerebral cavities.

Morphology

Toxoplasma is ovoidal in shape with one end attenuated and the other more rounded. It measures 2 to 4 microns in width by 4 to 7 microns in length. The nucleus is located near the rounded end. The centrosome or kinetoplast is not visible but in some specimens a chromatin dot lies between the nucleus and the attenuated end. Prior to cell division the parasite may appear oval rather than crescentic. There is no pigment. By the casual observer it may be confused with Leishmania.

Life cycle

At the present time the exact means of transmission is unknown. There is strong evidence that the disease is acquired by ingestion. However arthropods have been considered as possible vectors.

Laboratory diagnosis

Toxoplasma infections can be demonstrated by the presence of parasites in smear preparations, by immunological tests, by post mortem demonstration in tissues or by inoculation of laboratory mice with suspected tissues.

Sarcocystis lindemanni

This parasite occurs only sporadically in man and apparently is not a serious infection. Epidemiology of the parasite is unknown but Sarcocystis may be found as an elongated, cylindrical, tub-like (Miescher's tubes) body (up to 5cm.) in the muscle fibers of the diaphragm, tongue, chest, abdomen or myocardium. This hyaline cyst contains thousands of spore-like organisms each with a nucleus near the rounded end.

MEDICAL HELMINTHOLOGY

Helminthology is the study of parasitic worms (helminths) which are found on or in animals or plants. Medical Helminthology is restricted to the study of those helminths which parasitize man. These belong to four phyla of which two, the Platyhelminthes (flatworms) and Nemathelminthes (roundworms), are of importance.

The Phylum Platyhelminthes is the more primitive group. The great majority of the worms belonging to this phylum are flattened dorsoventrally, hence the common name, "flatworms." The phylum is divided into three classes as follows:

Class 1. Turbellaria - For the most part, these are free-living animals whose habitat is fresh and salt water.

Class 2. Trematoda - The members of this group are called flukes. All

trematodes are parasitic, some externally on aquatic animals and other internally on aquatic and land animals.

Class 3. Cestoidea - The members of this group are called tapeworms. With the exception of one primitive family living in the body cavity of ganoid fishes, tapeworms, in the adult stage, are parasites of the digestive tract of vertebrates and are profoundly modified for this kind of existence.

The Phylum Nemathelminthes includes worms of somewhat higher organization than that noted in flatworms. Most of them are elongated and cylindrical and are commonly known as threadworms or roundworms.

Class 1. Nematoda - Many of the members of this class are free-living forms, some of which are found in fresh or salt water and some in mud or in field or garden soil. About a dozen species are parasitic in man, some of them in the digestive tract and others in blood and tissues.

Phylum Gordiacea - These are popularly known as "horsehair snakes" or "hairworms" because of the idea, still existing, that they develop from horsehairs that fall into water. Occasionally they are accidentally swallowed with drinking water, but otherwise they are of no importance as human parasites.

Phylum Acanthocephala - Members of this group are known as spiny-headed worms. They are common parasites of all kinds of vertebrates but are particularly numerous in fishes and birds. Two species normally parasitic in other animals are occasionally found in man.

Scope of Present Discussion

The present discussion is limited to those classes which are most important in in Medical Helminthology - Trematoda and Cestoidea of the Phylum Platyhelminthes and the Nematoda of the Phylum Nemathelminthes.

A general taxonomic scheme of helminth parasites is presented on pages 100 and 101. Such a chart is used as a tool in expressing parasite groupings and may not necessarily be followed by all schools or workers in parasitology.

In order to avoid needless repetition, the laboratory methods employed in the diagnosis of helminth infections are discussed on pages 182-197.

PHYLUM PLATYHELMINTHES (FLATWORMS)

General Characteristics

Three body layers, bilaterally symmetrical, with bodies leaf- or band-shaped; mostly hermaphroditic, without body cavity, alimentary canal incomplete or entirely lacking; includes free-living and parasitic forms. The parasitic forms are known as flukes and tapeworms.

The flukes (Class Trematoda) are further characterized as follows: unsegmented bodies, oval or leaf-shaped; digestive system with a single opening which functions as both a mouth and an anus; exist as ectoparasites or endoparasites; mostly hermaphroditic; primarily parasites of vertebrates in the adult stage.

The tapeworms (Class Cestoidea) are ribbon- or band-shaped with bodies made up of a number of similar units called proglottides (singular, proglottis); without mouth or alimentary canal; hermaphroditic; adults almost exclusively parasitic in the alimentary canal of vertebrates.

FLUKES (CLASS TREMATODA)

Morphology of the Adult

Method of Attachment

All human flukes have two muscular suckers - one, the oral or anterior sucker surrounding the mouth and the other, the ventral sucker or acetabulum on the ventral surface (A and B Fig. 30).

Integument

The body is covered by a noncellular cuticula which is secreted by special cells embedded in the underlying tissue. It may be provided with rows of scale-like structures or spines.

Muscular System

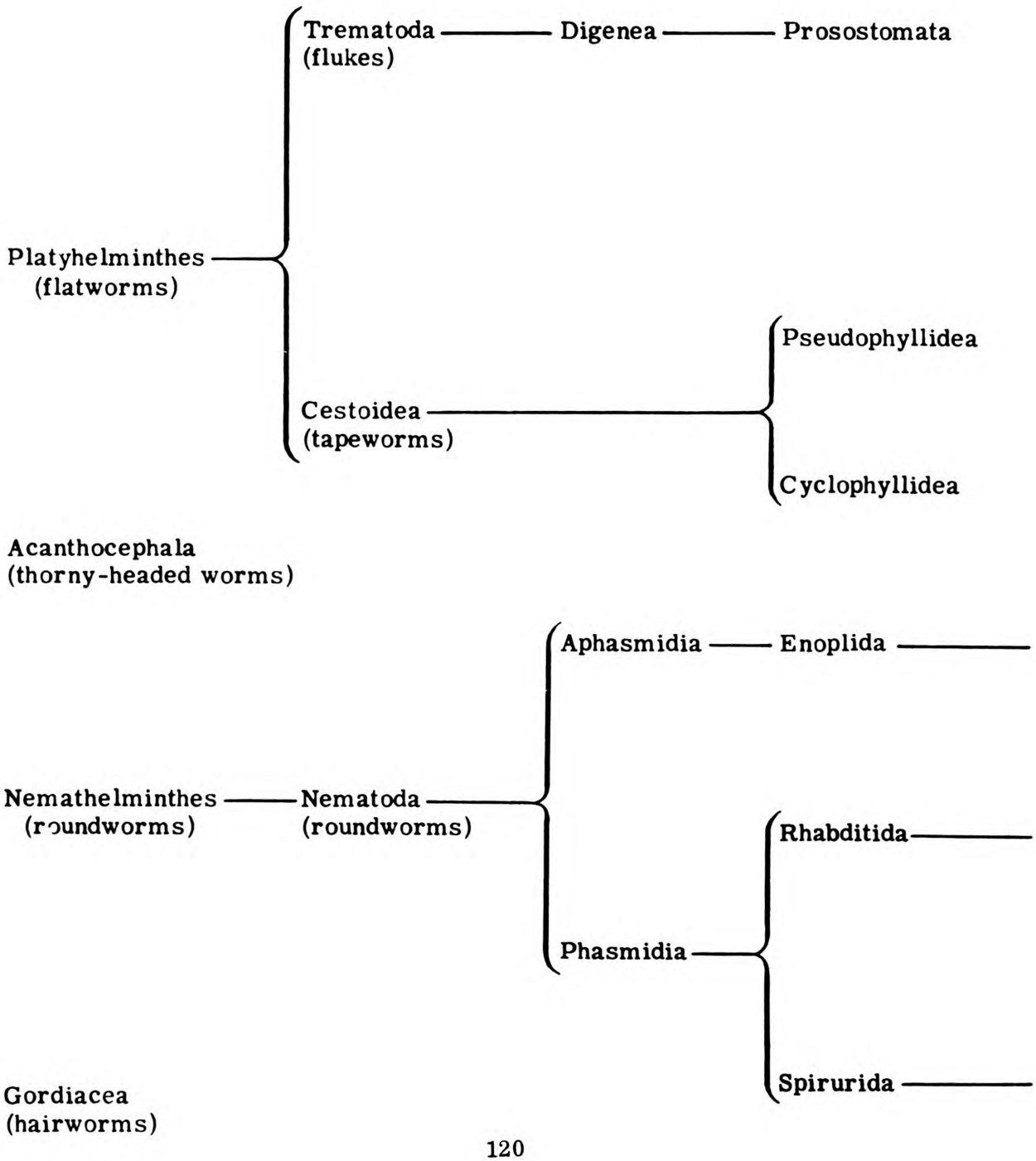
Three distinct layers of muscles, the outer circular, middle diagonal, and inner longitudinal, lie almost directly under the cuticula. Dorsoventral fibers are also present.

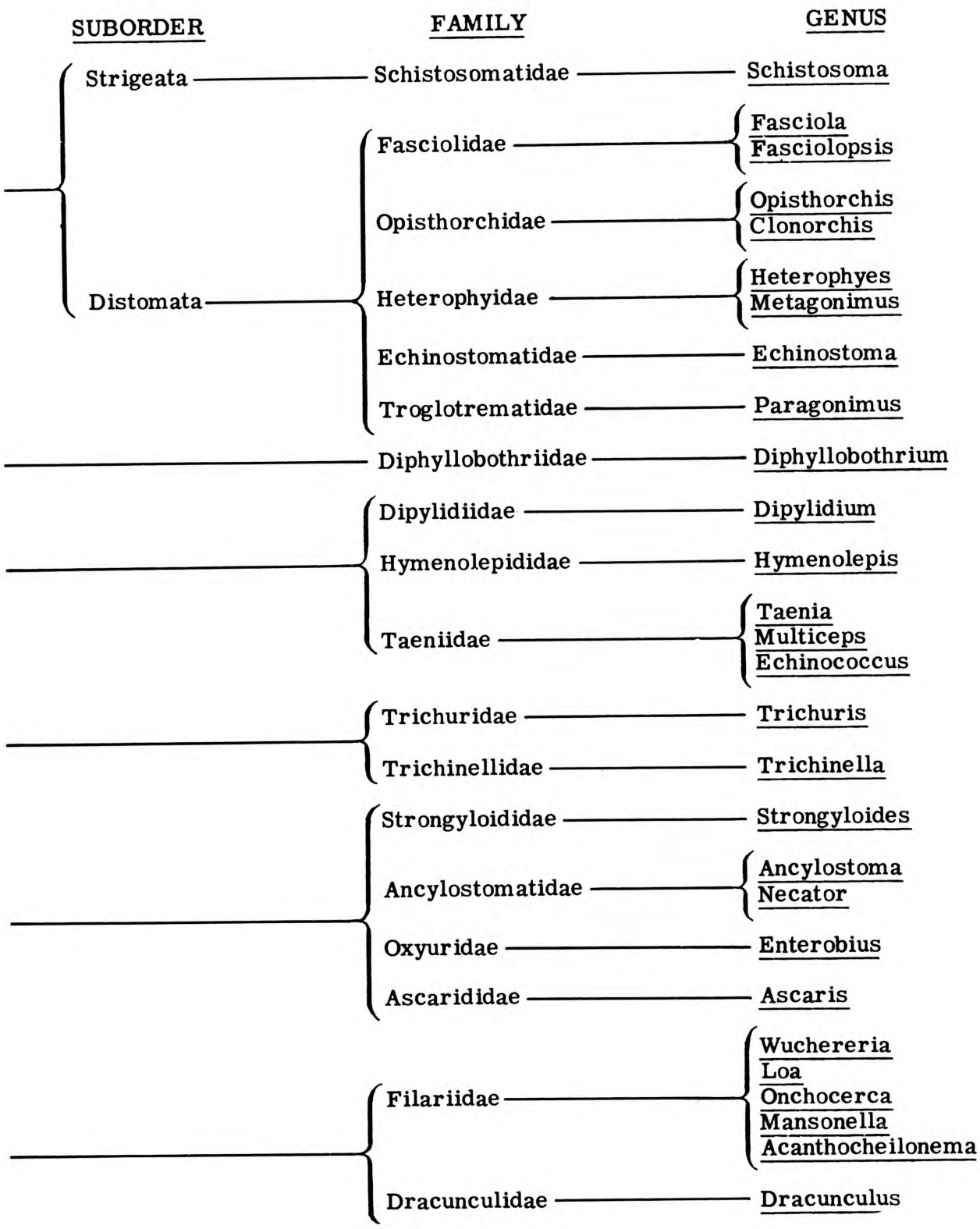
Nervous System (C Fig. 30)

Digestive System

This system consists of a mouth, muscular pharynx, esophagus, and digestive ceca.

CLASSIFICATION



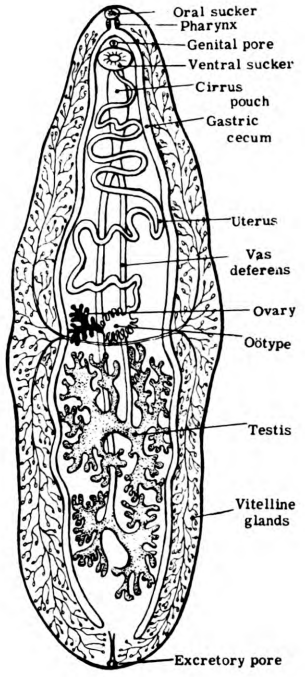

SUBORDER
FAMILY
GENUS
Strigeata
Schistosomatidae
Schistosoma
Distomata
Fasciolidae
Fasciola
Fasciolopsis
Opisthorchidae
Opisthorchis
Clonorchis
Heterophyidae
Heterophyes
Metagonimus
Echinostomatidae
Echinostoma
Troglotrematidae
Paragonimus
Diphyllobothriidae
Diphyllobothrium
Dipylidiidae
Dipylidium
Hymenolepididae
Hymenolepis
Taeniidae
Taenia
Multiceps
Echinococcus
Trichuridae
Trichuris
Trichinellidae
Trichinella
Strongyloididae
Strongyloides
Ancylostomatidae
Ancylostoma
Necator
Oxyuridae
Enterobius
Ascarididae
Ascaris
Filariidae
Wuchereria
Loa
Onchocerca
Mansonella
Acanthocheilonema
Dracunculidae
Dracunculus

Excretory System (D and G)

The basic unit of the excretory system is the flame cell located at the beginning of each of the excretory tubules. A network of tubes carries waste products to an excretory bladder which expels collected wastes to the outside by way of an excretory pore.

Reproductive System

Most flukes are hermaphroditic (monoecious) although a few, the blood flukes, are unisexual (dioecious). The male system (E, H, and I), consists of testes, usually two, and vasa efferentia which lead anteriorly from them and unite to form the vas deferens. The vas deferens expands into a cirrus pouch which envelops three structures, the seminal vesicle, the prostate glands, and the muscular cirrus or penis.



NAVAL MEDICAL SCHOOL

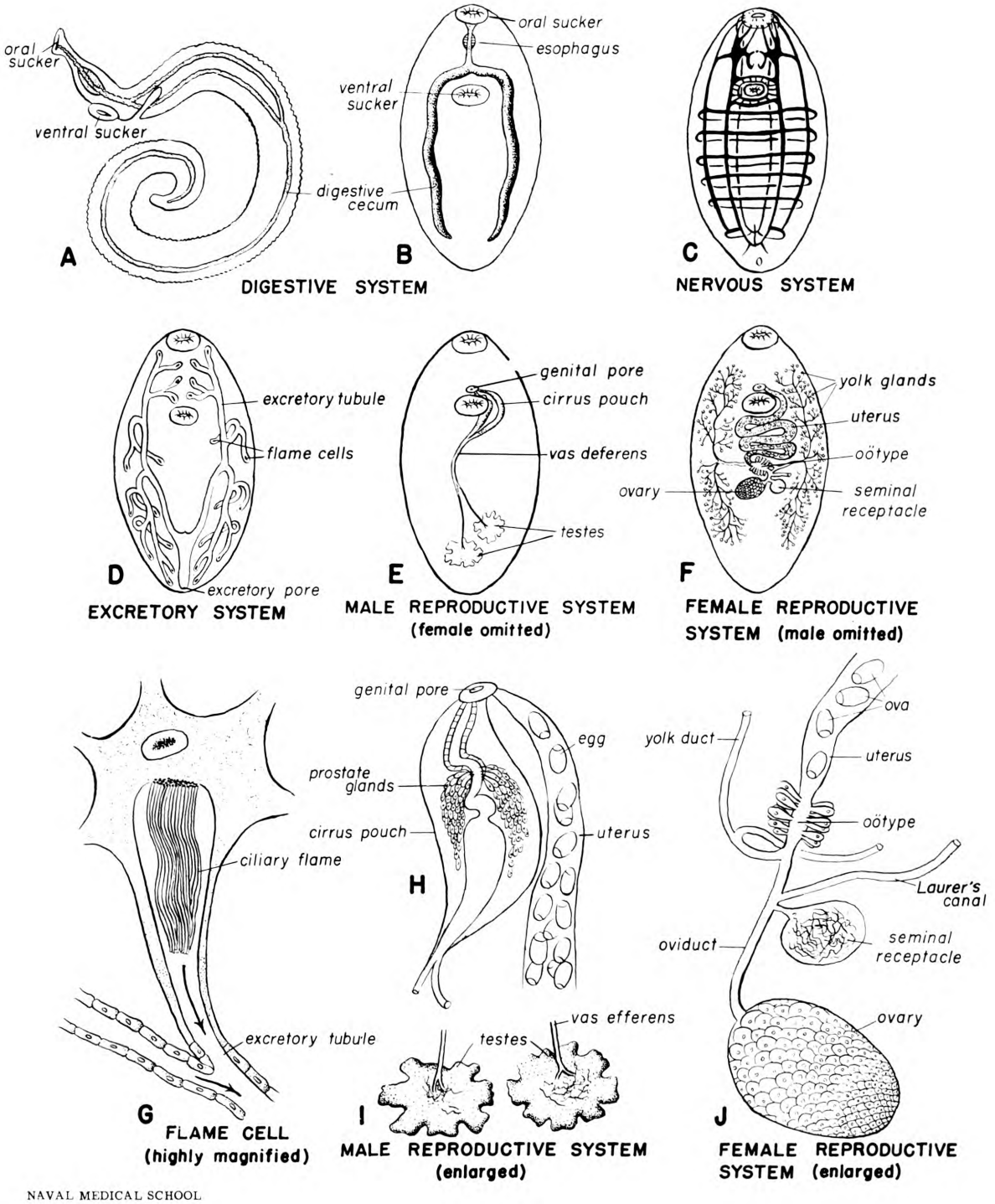
Fig. 29 - A fluke

The female system (F, H, and J) consists of a single ovary connected to the oviduct which dilates to form the oötype and then continues as the uterus. Associated with the oviduct are (1) the seminal receptacle in which the sperm cells are stored; (2) yolk ducts which carry nourishing material from the yolk glands or vitellaria; and (3) a duct, called Laurer's canal, which may end blindly or open to the surface. The oötype is muscular and its shape and size determine the shape and size of the egg. It is surrounded by cells (Mehlis' gland) which secrete the material utilized in the formation of the egg shell.

Morphology of the Immature States

The flukes have complicated but remarkably similar life cycles. Most of them are digenetic, i.e., there is an alternation of generations involving a definitive host, some vertebrate animal, in which the adult stage of the fluke is passed, and an intermediate host, usually a snail, in which the larval stages are passed. Many trematodes have a second intermediate host which serves merely as a vehicle for transferring the parasite from the first intermediate host (the snail) to the definitive host (man or other animals). This second intermediate host, in the case of human flukes, may be aquatic animals, such as fish, crabs, crayfish, or clams. In some species the second larval stage of the trematode may be deposited on vegetation (water cress, lettuce or other edible aquatic plants) rather than being transmitted by a second intermediate host. The flukes have several immature stages which are described and illustrated (Fig. 31).

MORPHOLOGY OF ADULT FLUKE



NAVAL MEDICAL SCHOOL

Fig. 30

Egg or Ovum

The egg or ovum is a germ cell (or multicellular mass) surrounded by yolk cells, enclosed in a shell. Fluke eggs may have either an operculum, a cap or lid which is forced off by the hatching embryo, or may be characterized by a specific type of spine. Although other helminths (lower tapeworms) may have operculate ova, spined eggs are found only in the flukes (schistosomes).

Miracidium

The miracidium is the ciliated larva which hatches from the egg. The egg may hatch in water or it may be swallowed by a snail and hatch in its intestine. Regardless of the place in which hatching takes place, the miracidium reaches the tissues of the snail by penetrating the external surface or the intestinal wall. It has a short, sac-like gut, several flame cells, and germ cells or germ balls which are destined to give rise to a new generation of organisms.

Sporocyst

The sporocyst is a nonciliated form produced by the metamorphosis of the miracidium within the snail. It is an irregular, sac-like structure containing germ balls which produce the next larval stage.

Daughter Sporocyst

The daughter sporocyst is a sporocyst arising from the germ balls of a primary (mother) sporocyst.

Redia

The redia is a larva which arises from the germ balls of a sporocyst. In a few instances rediae may develop from the germ balls of a miracidium. A redia is characterized by the possession of a mouth, muscular pharynx, and sac-like cecum. Germ balls bud off from the wall of the redia and give rise to the succeeding larval stage, the cercaria. A birth pore is present to allow the passage of daughter rediae or mature cercariae from the mother redia into surrounding tissues of the snail.

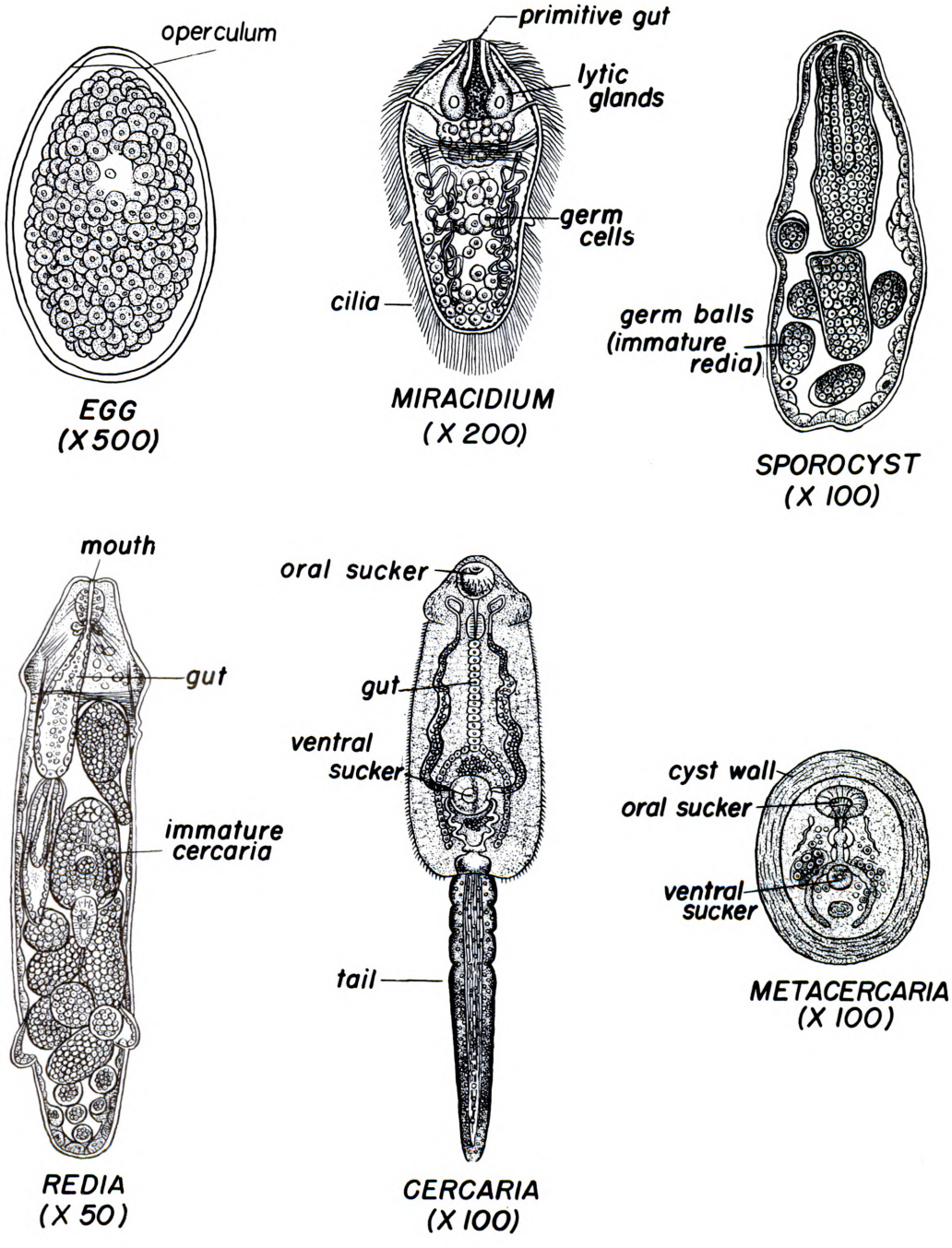
Daughter Redia

The daughter redia is a redia developed within a primary (mother) redia.

Cercaria

The cercaria is a larva arising from the germ balls of either a sporocyst or redia and characterized by having oral and/or ventral suckers, a mouth, pharynx, diverticulated gut, and usually a tail. This stage moves through and emerges from the snail's body. The cercaria swims about in the water until it encysts on vegetation or finds and penetrates the next host.

IMMATURE STAGES OF HUMAN FLUKES



NAVAL MEDICAL SCHOOL

Fig. 31

There are many kinds of cercariae which show a wide range in size and in morphology. A knowledge of cercarial types allows for tentative recognition of trematodes of a given family. Cercariae of the trematode parasites are commonly found in fresh, brackish and marine waters. The majority of species parasitzing man develop in fresh water mollusks.

Metacercaria

The metacercaria is the encysted stage of a cercaria. The cercaria attaches itself to a plant or animal, rounds up, loses its tail, and secretes a cyst wall about itself.

Life Histories of the Important Human Flukes

The adult fluke in man may live in the blood vessels (Schistosoma), in the liver (Fasciola, Clonorchis, Opisthorchis), in the intestine (Heterophyes, Metagonimus, Echinostoma, Fasciolopsis), or in the lungs (Paragonimus). The eggs laid in these sites pass out in the urine, feces, or sputum depending upon the location of the infection. As noted above, all ova except those of the schistosomes (blood flukes) have a cap or operculum which opens to allow the miracidium to escape. Each species of trematode requires a particular species of snail in which to continue its development. In many cases the miracidum is free-swimming, moves in a spirally rotating manner, and actively penetrates the soft parts of the snail by means of lytic glands, but the eggs of Heterophyes, Metagonimus, Opisthorchis, and Clonorchis must be ingested by an appropriate snail before they will hatch.

Upon gaining entrance into the tissues of the snail, the miracidium enters into the intramolluscan period of its existence. It reproduces enormously during this stage to compensate for the heavy mortality encountered during the passage from man (or other vertebrate) to the snail and to insure the safe arrival of at least a few individuals during the passage from the snail to man (or other vertebrate). The miracidium loses its ciliated coat and becomes transformed into a second larval stage which is usually a sporocyst but may be a redia. The distinctions between sporocysts, rediae, and cercariae are purely morphological and of little importance. Regardless of the number of larval generations within the snail, the last stage produced is a cercaria. The cercariae leave the snail and pursue various courses depending upon the species involved.

The schistosome cercariae actively penetrate the skin of man by means of lytic glands. The cercariae of Fasciola and Fasciolopsis encyst on vegetation; those of Clonorchis, Opisthorchis, Heterophyes, and Metagonimus in fish; those of Paragonimus in crayfish and crabs; and those of Echinostoma in a second mollusk. The encysted metacercariae in edible water plants, fish, crayfish, crabs, or mollusks enter man when he eats these foods insufficiently cooked or raw. The cercariae of the schistosomes and the excysted metacercariae of Paragonimus must

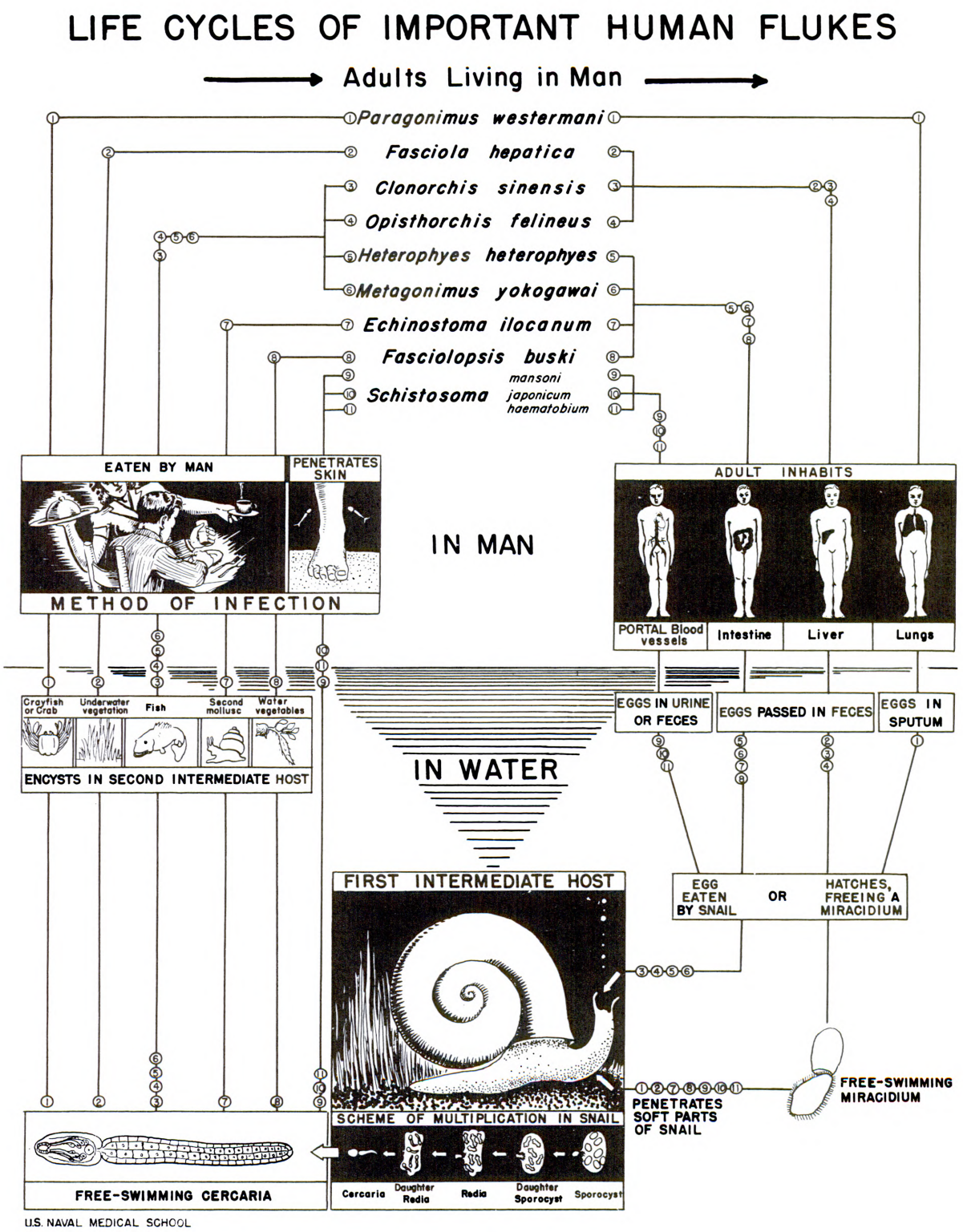

LIFE CYCLES OF IMPORTANT HUMAN FLUKES
Adults Living in Man
Paragonimus westermani
Fasciola hepatica
Clonorchis sinensis
Opisthorchis felineus
Heterophyes heterophyes
Metagonimus yokogawai
Echinostoma ilocanum
Fasciolopsis buski
Schistosoma mansoni japonicum haematobium
EATEN BY MAN
PENETRATES SKIN
METHOD OF INFECTION
IN MAN
ADULT INHABITS
PORTAL Blood vessels
Intestine
Liver
Lungs
Crayfish or Crab
Underwater vegetation
Fish
Second mollusc
Water vegetables
ENCYSTS IN SECOND INTERMEDIATE HOST
EGGS IN URINE OR FECES
EGGS PASSED IN FECES
EGGS IN SPUTUM
IN WATER
FIRST INTERMEDIATE HOST
EGG EATEN BY SNAIL
OR
HATCHES, FREEING A MIRACIDIUM
FREE-SWIMMING MIRACIDIUM
PENETRATES SOFT PARTS OF SNAIL
SCHEME OF MULTIPLICATION IN SNAIL
Cercaria
Daughter Redia
Redia
Daughter Sporocyst
Sporocyst
FREE-SWIMMING CERCARIA
U.S. NAVAL MEDICAL SCHOOL

Fig. 32

INTERMEDIATE HOSTS OF IMPORTANT HUMAN FLUKES

SPECIES	INTERMEDIATE HOSTS	
	First*	Second
BLOOD FLUKES		
Schistosoma haematobium	Bulinus, Planorbarius, Physopsis	
Schistosoma mansoni	Biomphalaria, Bulinus, Australorbis, Physopsis	None
Schistosoma japonicum	Oncomelania (includes Katayama and Schistosomophora)	
LIVER FLUKES		
Clonorchis sinensis	Thiara, Bulimus, Parafossarulus, Alocinma	Fish: minnows, carp, trout
Opisthorchis felineus	Bulimus (formerly Bithynia)	Fish: minnows, carp
Fasciola hepatica	Lymnaea, Bulinus, Succinea, Practicollela, Ampullaria	Aquatic vegetation
INTESTINAL FLUKES		
Fasciolopsis buski	Segmentina, Hippeutis	Aquatic vegetation, water chestnut, water caltrop
Heterophyes heterophyes	Pirenella, Tympanotonus	Fish: mullet, African perch
Metagonimus yokogawai	Semisulcospira, Cerithidea	Fish: trout
Echinostoma ilocanum	Gyraulus, Anisus	Snails, fresh-water clams
LUNG FLUKE		
Paragonimus westermani	Thiara (formerly Melania), Hua, Semisulcospira, Wanga, Assiminea	Crayfish, crabs

*Genera of snails

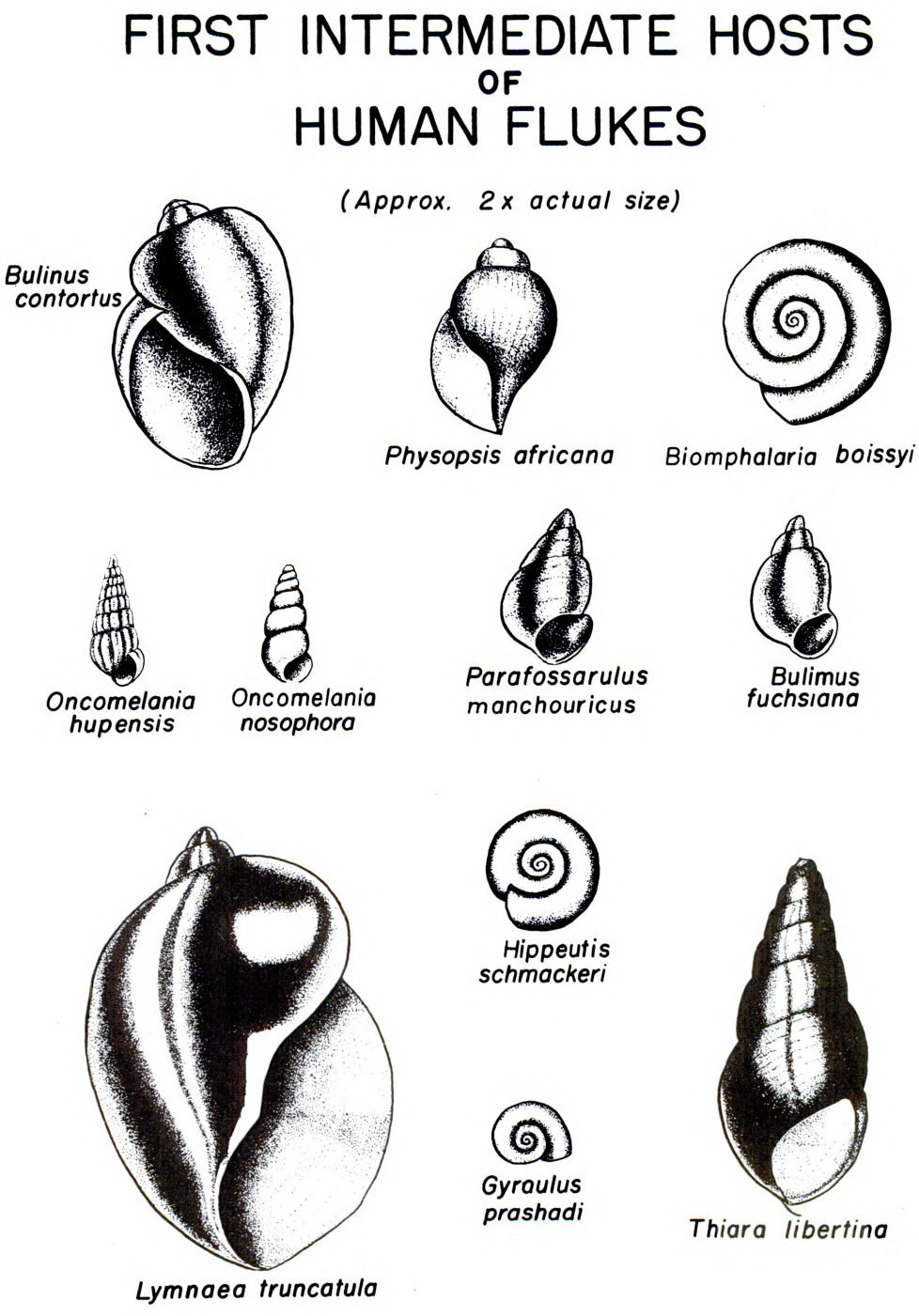

FIRST INTERMEDIATE HOSTS
OF
HUMAN FLUKES
(Approx. 2x actual size)
Bulinus contortus
Physopsis africana
Biomphalaria boissyi
Oncomelania hupensis
Oncomelania nosophora
Parafossarulus manchouricus
Bulimus fuchsiana
Hippeutis schmackeri
Gyraulus prashadi
Thiara libertina
Lymnaea truncatula
NAVAL MEDICAL SCHOOL

Fig. 33

undertake complicated migrations before developing to sexual maturity, while the intestinal and liver flukes undergo much less extensive migrations.

Cercarial Dermatitis

The cercariae of the schistosomes parasitizing birds and lower mammals as well as those found in man attack and are capable of readily penetrating the skin of humans. Non-human schistosomes as a rule do not survive or develop in man. Several hours after exposure to schistosome cercariae one may experience intense itching in the areas where the parasites have entered the skin, accompanied by edema and formation of papular eruptions which may become pustular. Reaction to invading cercariae becomes most intense three or four days after exposure. Dermatitis results from a sensitivity reaction. The condition or reaction may be expected to become more severe with repeated exposures to schistosome cercariae. Consequently sensitive persons should be warned not to swim or wade in water suspected of harboring snails from which fork-tailed schistosome cercariae are emerging.

Schistosome dermatitis is common in the north central lakes regions of the United States and Canada. Recent investigation has disclosed that this dermatitis is not restricted to fresh waters. Serious cases of dermatitis have been reported from the marine coastal waters of the northeastern United States, from California and from Hawaii. Schistosome dermatitis is found throughout Europe, Africa, parts of the Orient and probably in all countries in which lower mammal, bird or human schistosomes occur.

Schistosomiasis is the most important disease of man caused by helminths. It is also called bilharziasis in many regions of the world, after the generic synonym Bilharzia. Three species of Schistosoma are important parasites of man, and because of their similarities, they are considered together.

Geographic Distribution

Schistosoma haematobium

Africa	Prevalent in Egypt, Sudan, Ethiopia, Belgian Congo, Nigeria, Madagascar, Mauritius - actually found in the greater part of Africa.
Europe	Portugal, Spain, Cyprus
Asia	Palestine, Syria, Iraq, Mesopotamia, Arabia, India

Schistosoma mansoni

Africa	Egypt, Sudan, Rhodesia, Belgian Congo, Senegal, French Guinea, Sierra Leone, Madagascar - and greater part of Africa
South America	Brazil, Venezuela, Dutch Guiana
Caribbean Area	Puerto Rico, Windward and Leeward Islands

Schistosoma japonicum

Asia	China, Japan, Formosa(?)
Pacific Area	Philippines, Celebes

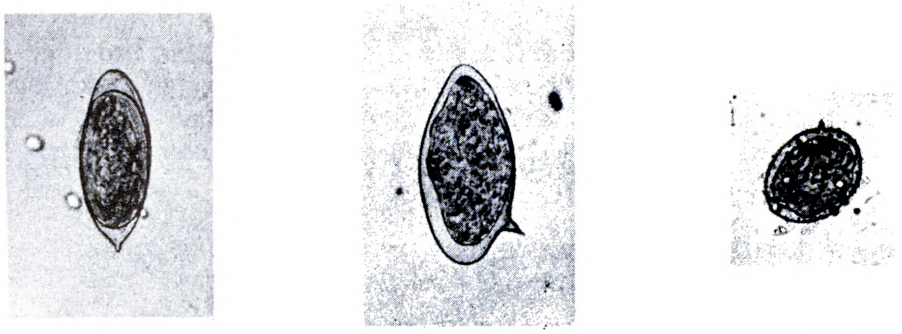


Fig. 34 - Eggs of the three important human schistosomes. Left, S. haematobium; center, S. mansoni; right, S. japonicum

Blood Flukes

Morphology of Adults

	S. japonicum	S. mansoni	S. haematobium
MALE			
Size (mm.)	9-22	6-14	10-18
Cuticle	Nontuberculate	Grossly tuberculate	Finely tuberculate
Testes	7	6-9	4
Reunion of Intestinal Ceca	In posterior fourth of body	In anterior half of body	About middle of body
FEMALE			
Size (mm.)	12-26	12-16	15-20
Number of Eggs in Uterus	50 or more	1-4	20 - over 100
Position of Ovary	Middle of body	Anterior body half	Posterior body half

Life Cycles
(Fig. 35)

1. The adults live in the venous plexuses of the bladder (S. haematobium), the colon (S. mansoni), or the small intestine (S. japonicum). Here the eggs are laid, and, by means of the cytolytic enzymes produced by the embryo and the body movements of the host, they penetrate the tissues and pass into the lumen of the small intestine (S. japonicum), the colon (S. mansoni, rarely S. haematobium), or the bladder (S. haematobium, rarely, S. mansoni).

2. The eggs usually mature before they pass in the urine or feces and hatch almost immediately in the water under favorable conditions.

3. The miracidia must penetrate the tissues of a suitable snail within a period of about thirty-two hours, after which time they are no longer infective.

4. Within the snail, two sporocyst generations give rise to the cercariae in about 1 month. The latter are produced continuously in small numbers by the daughter sporocysts for a period of several months, at the end of which time a single miracidium may have formed 100,000 to 200,000 cercariae.

5. There is no second intermediate host. Cercariae of the human schistosomes readily attack most mammals including man. Rodents, especially mice and hamsters, serve as satisfactory experimental hosts for work relating to schistosomiasis. Carnivores as a rule are extremely poor hosts or are non-susceptible to infection

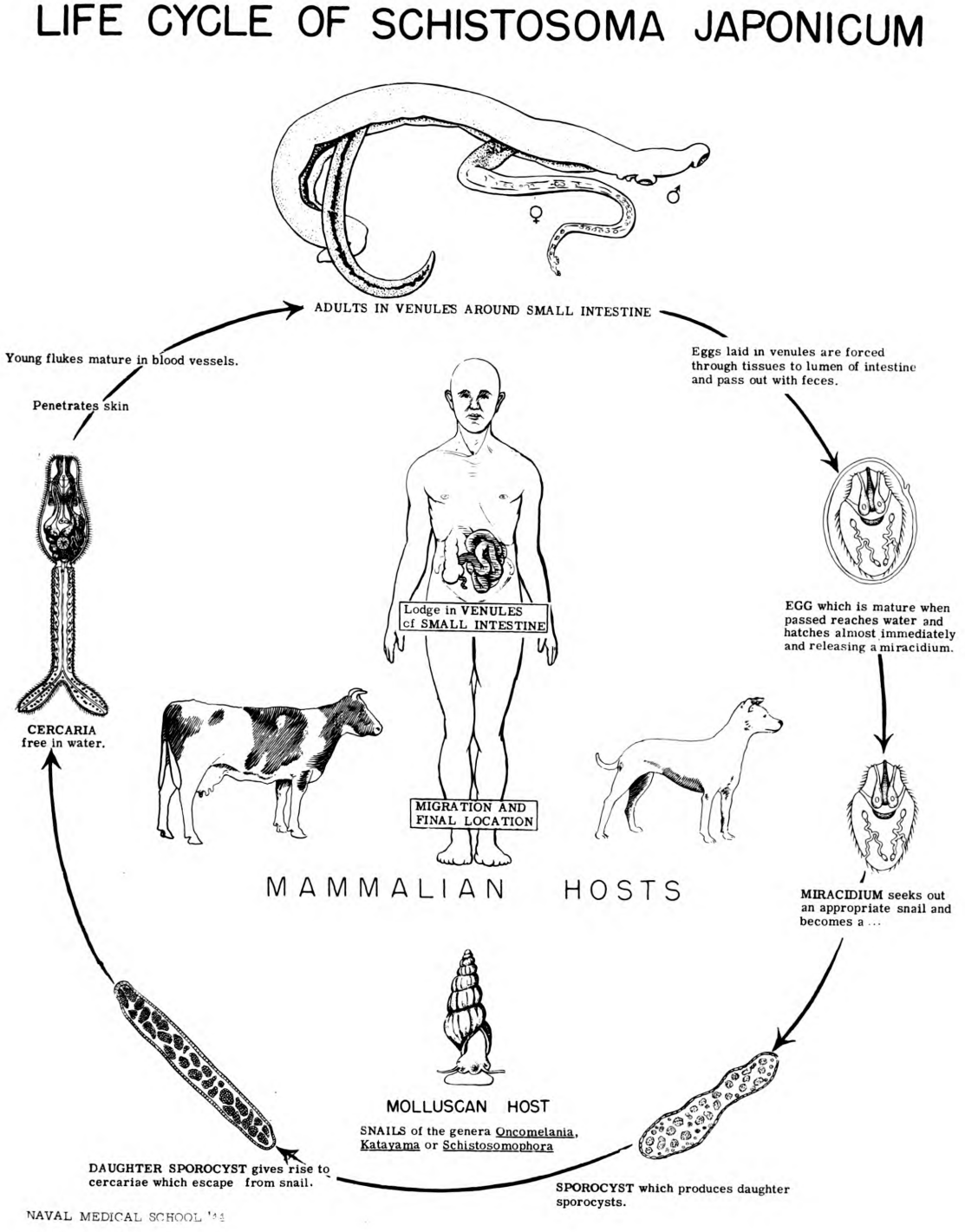

LIFE CYCLE OF SCHISTOSOMA JAPONICUM
ADULTS IN VENULES AROUND SMALL INTESTINE
Eggs laid in venules are forced through tissues to lumen of intestine and pass out with feces.
EGG which is mature when passed reaches water and hatches almost immediately and releasing a miracidium.
MIRACIDIUM seeks out an appropriate snail and becomes a ...
SPOROCYST which produces daughter sporocysts.
DAUGHTER SPOROCYST gives rise to cercariae which escape from snail.
CERCARIA free in water.
Penetrates skin
Young flukes mature in blood vessels.
Lodge in VENULES of SMALL INTESTINE
MIGRATION AND FINAL LOCATION
MAMMALIAN HOSTS
MOLLUSCAN HOST
SNAILS of the genera Oncomelania, Katayama or Schistosomophora
NAVAL MEDICAL SCHOOL '44

Fig. 35

by S. mansoni and S. haematobium. S. japonicum, on the contrary, is capable of effectively parasitizing carnivores, rodents and a number of domestic animals including cats, dogs, pigs and cattle. Schistosome cercariae are rather fragile but may remain infective for two days under optimal conditions.

LIVER FLUKES

Clonorchis sinensis

This parasite is very important, being responsible for heavy infection in certain oriental areas where raw fish is a common article of food.

Geographic Distribution

Japan, Korea, all of China except the northwest, Formosa, and French Indo-China.

Morphology of Adult

This fluke is a flat, transparent, flabby, spatulate organism, attenuated anteriorly and somewhat rounded posteriorly. It is 10 to 25 mm. long and 3 to 5 mm. wide. The oral sucker is slightly larger than the ventral sucker which is situated at the posterior end of the anterior third of the body. The intestinal ceca are unbranched and extend to the posterior end of the body. The testes are deeply lobulated or branched and are situated one behind the other in the posterior third of the body.

Life Cycle (Fig. 37)

1. The eggs are very resistant and may remain viable up to 6 months. Although the ova are mature when they pass out of the body, the miracidia hatch only after being ingested by certain snails.

2. The cercariae must find certain fish within 24 to 48 hours or die.

3. The metacercariae excyst in the gut of man, dogs, cats, hogs, wildcats, martens, badgers, or minks.

4. In the duodenum, the excysted larva migrates to the opening of the bile duct and up into the distal bile passages.

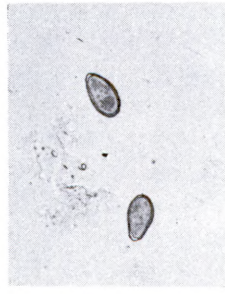


Fig. 36 - Eggs of Clonorchis sinensis

Opisthorchis felineus

This is a common parasite in certain areas in East Prussia and Siberia where fish are eaten uncooked.

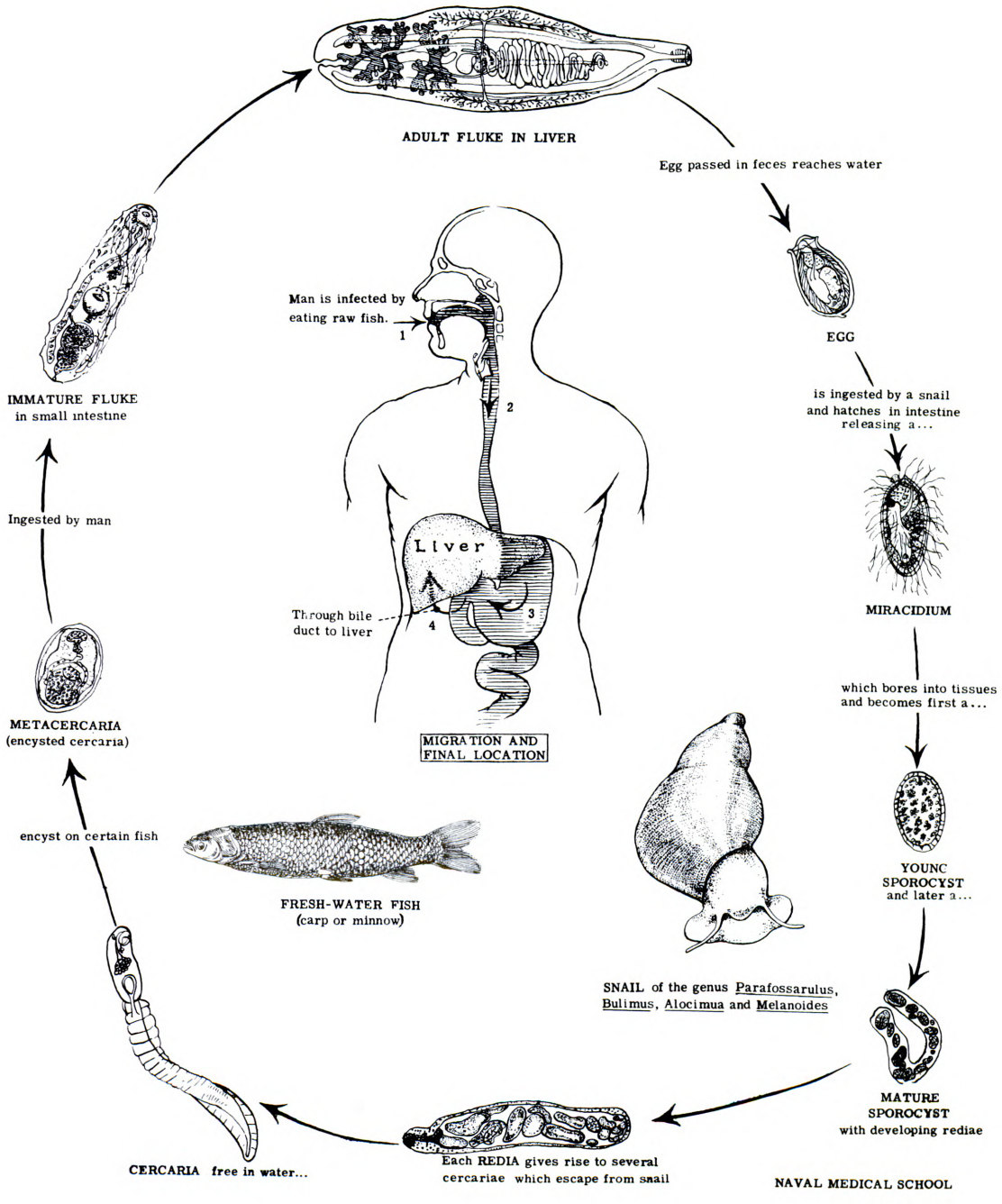

LIFE CYCLE OF CLONORCHIS SINENSIS
ADULT FLUKE IN LIVER
Egg passed in feces reaches water
EGG
is ingested by a snail and hatches in intestine releasing a...
MIRACIDIUM
which bores into tissues and becomes first a...
YOUNG SPOROCYST and later a...
MATURE SPOROCYST with developing rediae
Each REDIA gives rise to several cercariae which escape from snail
CERCARIA free in water...
encyst on certain fish
METACERCARIA (encysted cercaria)
Ingested by man
IMMATURE FLUKE in small intestine
Man is infected by eating raw fish.
1
2
Liver
3
4
Through bile duct to liver
MIGRATION AND FINAL LOCATION
FRESH-WATER FISH (carp or minnow)
SNAIL of the genus Parafossarulus, Bulimus, Alocimua and Melanoides
NAVAL MEDICAL SCHOOL

Fig. 37

Geographic Distribution

Prussia, Siberia, India, Japan, and French Indo-China.

Morphology of Adult

The adult worm is lancet-shaped, rounded posteriorly and attenuated anteriorly, thin and transparent, and measures 7 to 12 mm. in length and 2 to 3 mm. in breadth. The two suckers are nearly equal in diameter (0.25 mm.); the oral sucker is subterminal, and the ventral sucker is situated about one-fifth the body distance from the anterior end. The intestinal ceca are unbranched and extend to the posterior end of the worm. The two testes are lobed and are situated obliquely to each other in the posterior fourth of the worm.

Life Cycle (Fig. 32)

It is similar to that of C. sinensis (Fig. 37). Opisthorchis viverrini, similar to O. felineus, has recently been found to be a rather common parasite in certain parts of Thailand.

Fasciola hepatica

Although this species was the first trematode to be described and also the one for which the first complete life cycle of a digenetic fluke was elucidated, it is of minor importance as a parasite of man.

Geographic Distribution

Cosmopolitan; it is prevalent in sheep-raising countries.

Morphology of Adult

Fasciola hepatica is a fleshy fluke measuring up to 30 mm. in length by 13 mm. in width; the margins are relatively flat and slightly convoluted. At the anterior end there is a distinct conical projection. The posterior end is broadly pointed. The oral sucker is about 1 mm. in diameter, and the ventral sucker, which is nearby, is 1.6 mm. in diameter. The intestinal ceca are branched and extend to the posterior end of the worm. The two testes, which lie one behind the other in the second and third fourths of the body, are conspicuously branched.

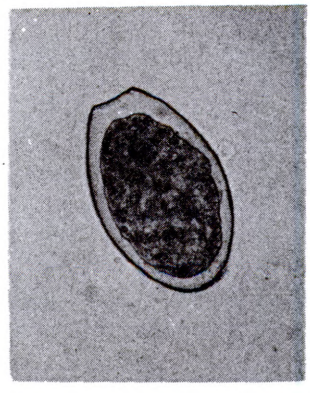


Fig. 38 - Egg of Fasciola hepatica (operculum detached)

Life Cycle (Fig. 32)

It is similar to that of F. buski (Fig. 40) and differs from those of Clonorchis and Opisthorchis in the following points:

1. The eggs must develop to maturity in water where they remain viable up to 9 months or more.

2. The miracidia hatch in the water and penetrate the tissues of an appropriate snail.

3. A second or daughter redial generation is added.

4. The cercariae emerge from the snail during mid part of day, and the majority encyst on aquatic vegetation within 1/2 to 3 hours.

5. The metacercariae excyst in the gut of man, horses, sheep, camels, goats, llamas, elephants, beavers, deer, antelopes, and monkeys.

6. After excysting in the duodenum of the definitive host, the immature fluke penetrates the intestinal wall and migrates to the liver.

INTESTINAL FLUKES

Heterophyes heterophyes

This parasite is common in man in certain regions of the world, but it usually does not produce serious disease.

Geographic Distribution

Egypt, Palestine, Japan, China, Korea, Formosa, and the Philippine Islands.

Morphology of Adult

A very small trematode, measuring from 1.0 to 1.7 mm. in length by 0.3 to 0.7 mm. in breadth. It is oval and elongate in shape and gray in color when passed in fresh feces; a brown spot marking the position of the uterus is visible on the mid-ventral surface. The oral sucker is subterminal and the ventral sucker is located immediately in front of the mid-body. A sucker-like structure, known as the gonotyle, surrounds the genital pore. The intestinal ceca are unbranched and extend to the posterior end of the body.

Life Cycle (Fig. 32)

Metagonimus yokogawai

This fluke is very common in Japan and is more prevalent and more widely distributed in that country than C. sinensis.

Geographic Distribution

Japan, Korea, China, Formosa, Dutch East Indies, the Balkans, and Palestine.

Morphology of Adult

This fluke resembles H. heterophyes in size and shape. The distinctive features involve the ventral sucker which is 66 to 165 microns long, 55 to 114 microns wide, and situated to one side of the mid-line with its long axis in a diagonal plane. Closely associated with the ventral sucker is the genital opening, its muscular outer rim fused with the ventral sucker.

Life Cycle (Fig. 32)

1. Under favorable conditions, the eggs remain viable for 6 months or more.

2. The cercariae are viable for only a few hours after leaving the snail.

3. The metacercariae excyst in the gut of man, cats, dogs, and foxes.

Fasciolopsis buski

This species is an important parasite of man in Asia. In certain regions of China it is so prevalent that it is one of the principal causes of illness and results in considerable loss of life.

Geographic Distribution

Russia, China, Siam, India, Borneo, Sumatra, and Malay Peninsula.

Morphology of Adult

F. buski is the largest trematode parasite of man and may attain a length of 75 mm. with a width of 15 mm. The average length is about 30 mm., average width about 12 mm., and average thickness about 2 mm. The cuticula is covered with small, backward-pointing spines arranged in transverse rows. The oral sucker is subterminal on the ventral side and is about 0.5 mm. in diameter. The ventral sucker lies posterior and close to the oral sucker; it is from two to three times as large as the oral sucker and has a pear-shaped opening. The intestinal ceca, arising almost directly from the pharynx, are unbranched and pass to the posterior extremity with two characteristic curves, one at the anterior border of the anterior testis, the other between the testes. The testes which are multi-branched occupy the posterior half of the body within the central area and lie one behind the other.

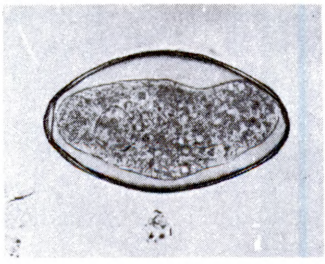


Fig. 39 - Egg of Fasciolopsis buski

Life Cycle (Fig. 40)

1. Under favorable conditions, the eggs remain viable for 6 months or longer.

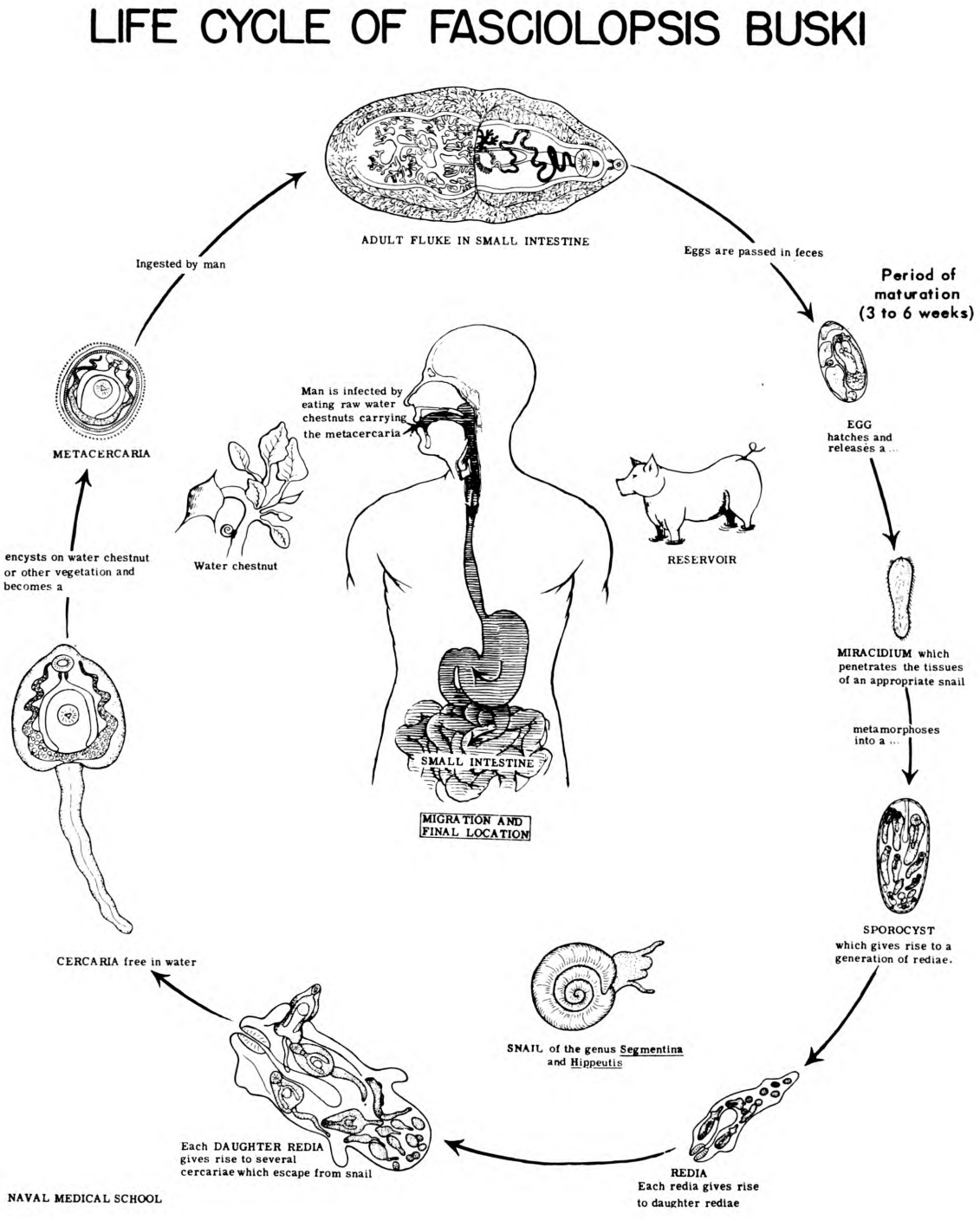

LIFE CYCLE OF FASCIOLOPSIS BUSKI
ADULT FLUKE IN SMALL INTESTINE
Eggs are passed in feces
Period of maturation (3 to 6 weeks)
EGG hatches and releases a ...
MIRACIDIUM which penetrates the tissues of an appropriate snail
metamorphoses into a ...
SPOROCYST which gives rise to a generation of rediae.
REDIA Each redia gives rise to daughter rediae
SNAIL of the genus Segmentina and Hippeutis
Each DAUGHTER REDIA gives rise to several cercariae which escape from snail
CERCARIA free in water
encysts on water chestnut or other vegetation and becomes a
METACERCARIA
Ingested by man
Water chestnut
Man is infected by eating raw water chestnuts carrying the metacercaria
RESERVOIR
SMALL INTESTINE
MIGRATION AND FINAL LOCATION
NAVAL MEDICAL SCHOOL

Fig. 40

2. The cercariae are viable for only a few hours after leaving the snail.

3. The metacercariae excyst in the gut of man and hogs.

LUNG FLUKE

Paragonimus westermani

This parasite was first discovered in the lungs of tigers in 1877. A few years later (1883) it was found in the lungs of man.

Geographic Distribution

The Orient, Philippines, South America, and Africa; possibly cosmopolitan in the lower animals.

Morphology of Adult

The living specimens have no very definite shape. When killed and preserved, they have an oval or elliptical form resembling coffee beans in both size and shape. They measure 7.5 to 12 mm. in length, 4 to 6 mm. in breadth, and 3.5 to 5 mm. in thickness. The integument is provided with scale-like spines which cover the worm. The oral sucker is situated at the anterior extremity of the body and is 0.7 to 0.8 mm. in diameter. The ventral sucker is situated somewhat anteriorly to the middle of the body and often lies completely invaginated, leaving a relatively narrow external opening visible from the body surfaces. The intestinal ceca are unbranched and extend nearly to the posterior end of the worm. The testes are deeply lobed and are situated nearly side by side midway between the ventral sucker and the posterior end.

Life Cycle (Fig. 42)

1. The eggs are immature when passed in sputum or feces; from 4 to 6 weeks in water may be required for the miracidium to become mature and escape from its shell.

2. The miracidium must penetrate into the tissues of an appropriate snail within twenty-four hours after escaping from the egg.

3. The metacercariae excyst in the gut of man, dogs, cats, wildcats, tigers, mountain lions, foxes, martens, badgers, minks, rats, muskrats, weasels, wolves, goats, and hogs.

Fig. 41 - Egg of Paragonimus westermani

4. Approximately 30 days are required for the migration of the immature fluke from the intestine through

LIFE CYCLE OF PARAGONIMUS WESTERMANI

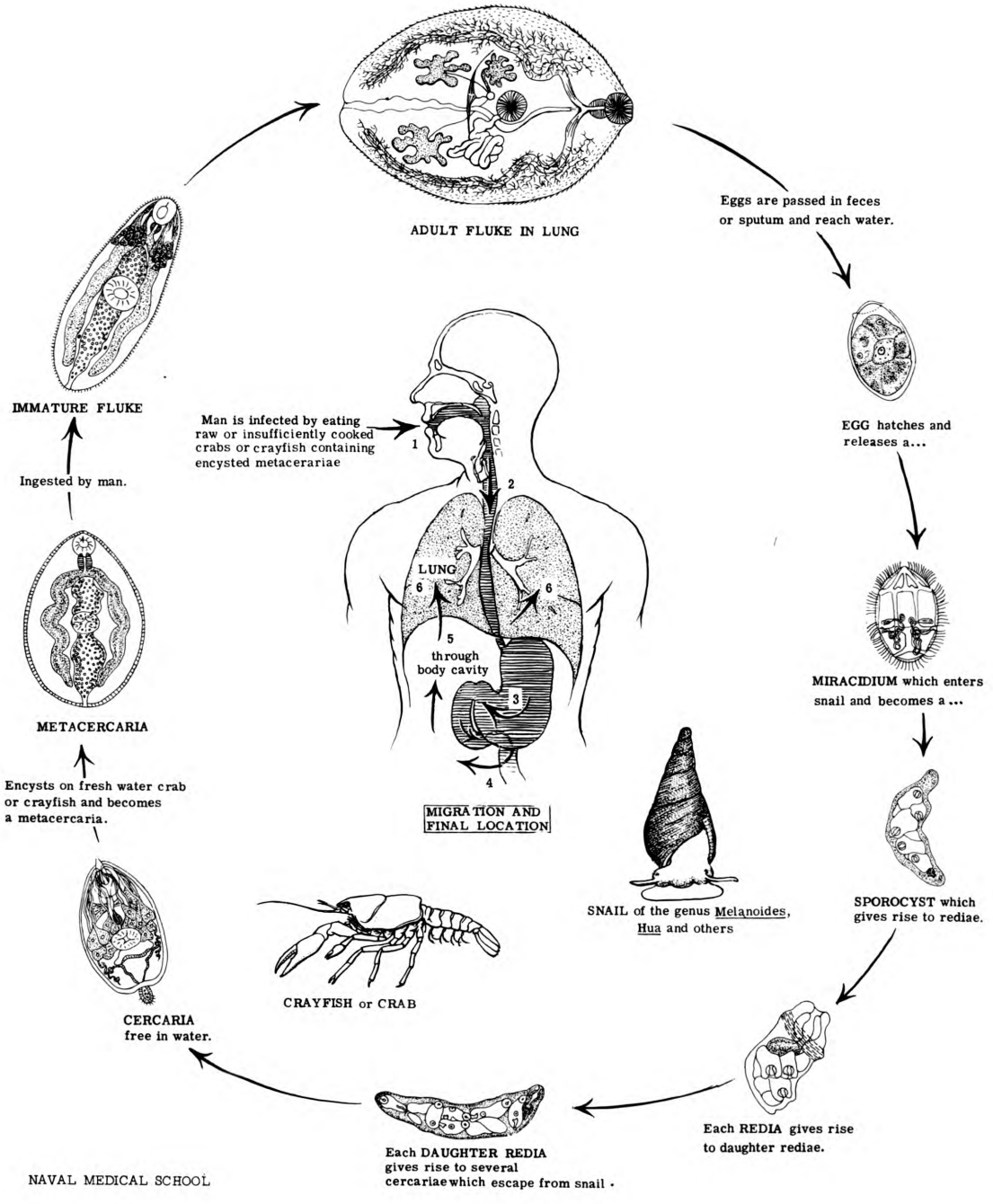


Fig. 42

the peritoneal cavity, diaphragm, and pleural cavity to the lung.

P. kellicotti, is a closely related species which is found as a parasite of carnivores and other mammals in the United States. The definitive host is infected by ingestion of infected fresh water crayfish (Cambarus). A small amphibious snail (Pomatiopsis) serves as the first intermediate host. This lung trematode has been reported from man.

LABORATORY DIAGNOSIS OF FLUKE INFECTIONS

The laboratory diagnosis of trematode infections depends almost entirely upon the finding and identification of the characteristic ova. All of the eggs may be passed in the feces, some of them being found exclusively in the contents of the intestinal tract. Of the others, eggs of Paragonimus westermani normally appear in the sputum but are swallowed and found also in the feces in nearly one-half of the cases; eggs of Schistosoma haematobium are passed in the urine, rarely in the feces; and those of S. mansoni may very occasionally be found in the urine.

Feces should be examined by the direct smear, sedimentation, and centrifugation technics. Flotation methods may be helpful in increasing the recovery of eggs but are not always successful: The ova of blood flukes become distorted; the large operculate eggs explode their caps and become unrecognizable; and the small operculate eggs are too heavy to be floated by the solution used. Hatching technics may be used for any of the ova containing a miracidum and have been found to give excellent results with the schistosomes. The acid-ether technic is also highly recommended for the eggs of S. mansoni and S. japonicum.

The examination of urine employs essentially the same methods as used for feces; the last portion voided contains most of the eggs. In the case of S. haematobium infection greater numbers of eggs may be found in urine collected following a period of vigorous exercise. Sputum is usually examined directly or after centrifugation; in paragonimiasis it contains minute iron-brown flecks formed by masses of ova and usually altered blood, eosinophils, and Charcot-Leyden crystals. The ova of liver flukes may be numerous in the bile when they are scarce in the feces.

Certain factors affect the occurrence of ova in the excreta. Early infections, before the adults are mature, obviously will not exhibit eggs. Unisexual infection by the blood flukes also does not show ova; such cases may not be rare, especially in individuals whose infection has resulted from a short exposure in the endemic area. Scarring and hyperplasia of tissues, as seen in the blood flukes may prevent the ova from passing into the excreta; the number of eggs may be reduced or at least influenced by aging and death of the adults. Ova may occur in showers; it was found that U.S. Navy personnel infected with S. japonicum on Leyte Island would show ova in their stools in considerable numbers one day and then none for several weeks.

The great majority of eggs can be distinguished by the characters given in the following chart; they may also be identified by the use of the key (page 183). Several cautions should be interjected; The eggs of Fasciolopsis buski and those of Fasciola hepatica are practically indistinguishable; Clonorchis and Opisthorchis ova are very similar, also those of Heterophyes and Metagonimus; they can be identified only after careful study. Finally, the operculate immature eggs of the pseudophyllidean tapeworms must not be confused with the somewhat similar ova of Paragonimus and Echinostoma.

DIFFERENTIAL CHARACTERS OF TREMATODE EGGS

SPECIES	SIZE (in microns)	SPECIAL FEATURES
Schistosoma japonicum	70-105 x 55-80; average 85 x 60	Nonoperculate; inconspicuous, lateral, curved spine, often not seen; contains miracidium
Schistosoma mansoni	110-180 x 45-75; average 155 x 65	Nonoperculate; conspicuous lateral spine; contains miracidium
Schistosoma haematobium	110-170 x 40-75; average 150 x 60	Nonoperculate; conspicuous terminal spine; contains miracidium
Clonorchis sinensis	27-35 x 12-20; average 29 x 16	Distinctly operculate; contains asymmetrical miracidium; light bulb-shaped; shoulders
Opisthorchis felineus	Average 30 x 12	Distinctly operculate; contains asymmetrical miracidium; shoulders not pronounced
Opisthorchis viverrini	Average 26 x 13	Distinctly operculate; contains asymmetrical miracidium
Fasciola hepatica	130-150 x 65-90; average 140 x 80	Indistinctly operculate; immature; yolk granules concentrated around nuclei of cell
Fasciolopsis buski	130-140 x 80-90; average 135 x 82	Indistinctly operculate; immature; yolk granules evenly distributed in yolk cells
Heterophyes heterophyes	28-30 x 15-17; average 29 x 16	Distinctly operculate; contains symmetrical miracidium; shoulders not pronounced
Metagonimus yokogawai	26-28 x 15-17; average 27 x 16	Distinctly operculate; contains symmetrical miracidium; shoulders not pronounced
Echinostoma ilocanum	85-115 x 55-70; average 90 x 60	Indistinctly operculate; immature when passed
Gastrodiscoides hominis	150-170 x 60-70; average 160 x 65	Distinctly operculate; immature; rhomboidal, tapering toward poles
Paragonimus westermani	75-120 x 45-65; average 95 x 55	Distinctly operculate; golden-brown; immature; shoulders distinct but not pronounced

Special tests are of value in schistosomiasis when the diagnosis cannot be confirmed by demonstrating ova; they may also be used as a criterion of cure. Complement fixation, precipitin, and skin tests have been used by a number of investigators with excellent results; the skin test is perhaps the best aid since it combines ease of performance with accuracy. As euglobulin is increased in schistosomiasis, the aldehyde and similar tests (page 88) may be used; other diseases which produce this change must be excluded. Recently it has been found that biopsy of the rectal ampulla gives the highest number of positives in infection with S. mansoni; biopsy of the liver the next highest number, and stool examinations the lowest rate of infection. A single provocative injection of 1.0 gm. Bayer 205 may cause the ova of S. haematobium to appear in the urine within 18 hours in latent cases.

Adult flukes may be recovered at autopsy, or intestinal flukes when passed in the feces after treatment, and identified by their characteristic structure (see key, page 182).

Morphology of the Adult

An adult tapeworm is composed of three fairly distinct regions: (1) the scolex, (2) the neck, and (3) the strobila or the chain of proglottides. The scolex is more or less enlarged, globular or oval in outline, and it is structurally adapted for attachment to the intestinal wall. Immediately behind the scolex there is usually a slight constriction which is termed the neck. This is the budding zone composed largely of germinative tissue from which new proglottides are formed. The new

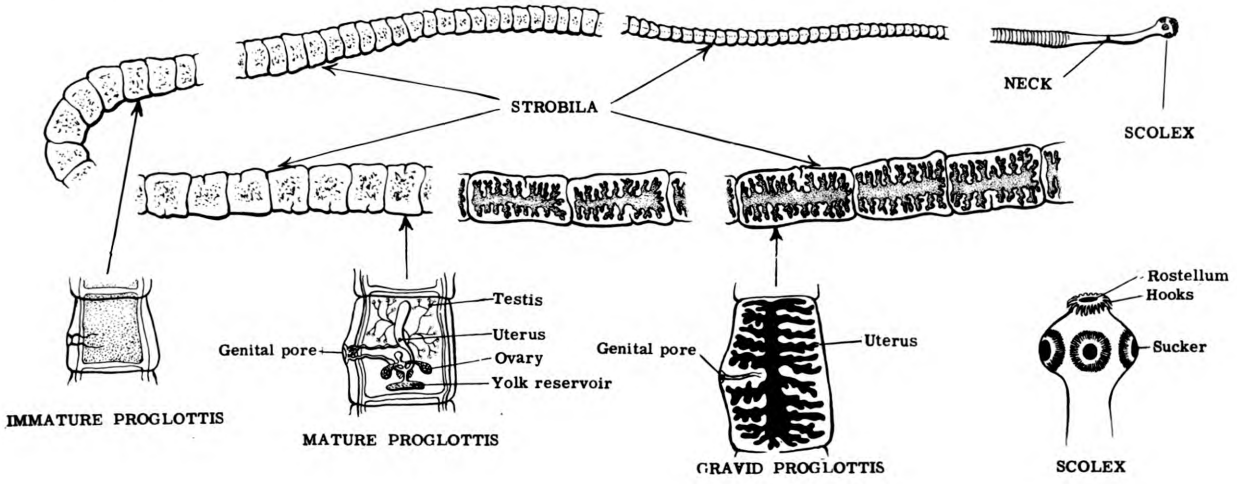


Fig. 43 - A tapeworm

segments are budded off at the posterior portion of the neck and, as they are pushed posteriorly by the new proglottides, they gradually develop. The proglottides may be grouped more or less into regions according to their maturity. Thus there is an immature region near the neck, a mature region near the middle, and a gravid region at the posterior end, each region consisting of immature, mature, and gravid proglottides respectively.

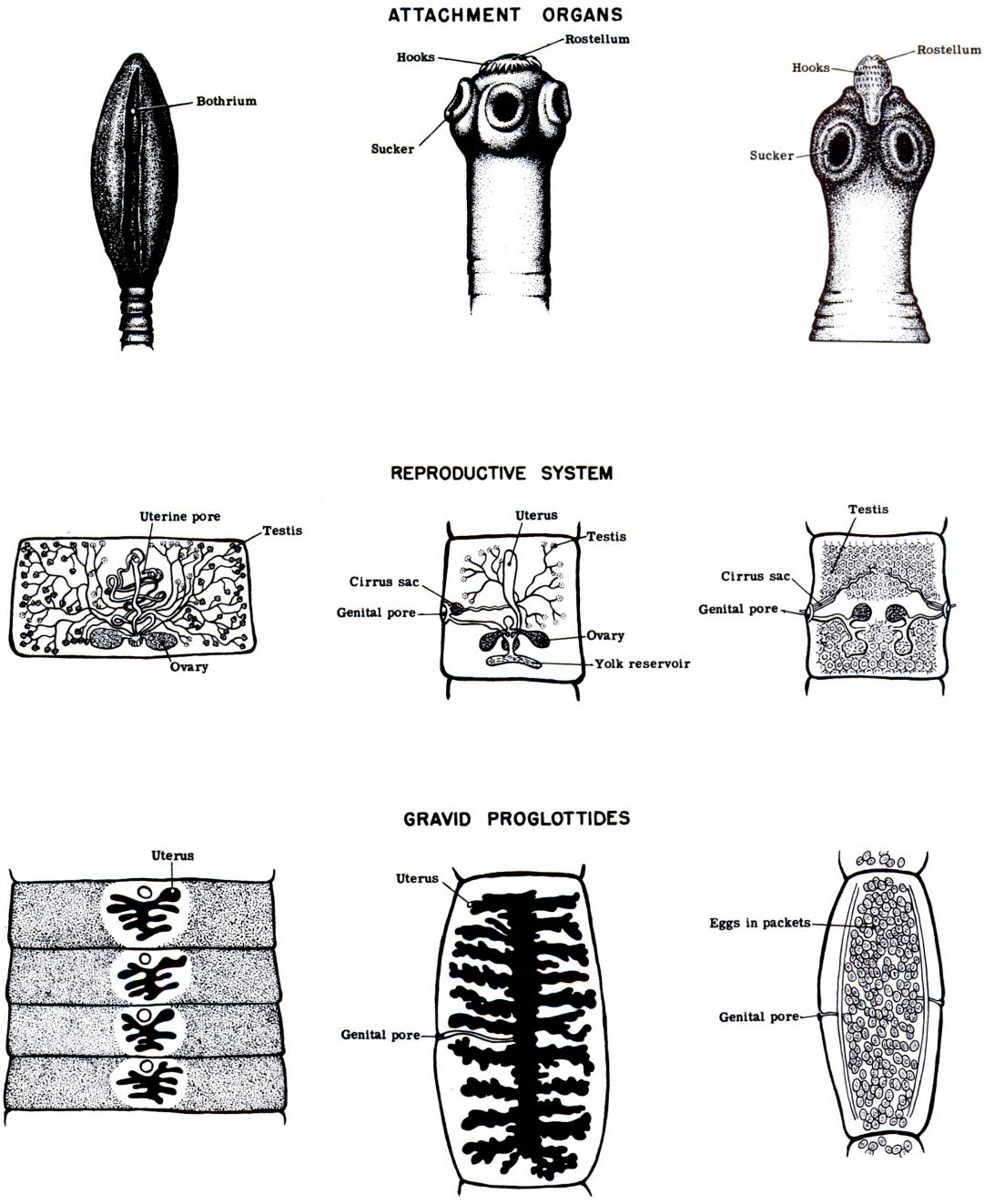
Method of Attachment

Note the two types of scolices. In the false suckered tapeworms (Order Pseudophyllidea) there are suctorial grooves, while in the round suckered cestodes (Order Cyclophyllidea) there are cup-shaped suckers which serve as organs of attachment.

Integument

The body is covered by a homogeneous, elastic cuticle similar to that of flukes.

MORPHOLOGY OF ADULT TAPEWORM



NAVAL MEDICAL SCHOOL

Fig. 44

Muscular System

This is highly developed and consists of circular, longitudinal, transverse, and dorsoventral fibers.

Nervous System

Similar to that of the trematodes with the center located in the scolex and three pairs of nerve trunks in the laterial fields of each proglottid.

Excretory System

A tubular system with flame cells but there is no excretory bladder. There are two ventrolateral and two dorsolateral canals running the length of the parasite. The longitudinal tubes are joined by anastomoses in the scolex and by a transverse canal at the posterior margin of each proglottid.

Digestive System

This system is absent.

Reproductive System

Two rather divergent types of reproductive system are represented in the tapeworms. The first type is seen in Diphyllobothrium and its relatives where two genital openings are found on the flat surface of the worm, one the male-female gonopore and the second the uterine pore. Eggs are laid continuously by the mature proglottides. In the second type found in all the other tapeworms of man, a single or sometimes double genital pore is present on the side of the proglottis. The uterine pore has disappeared entirely, and the eggs cannot be laid; instead, they remain in the uterus, dilating it in various patterns in Taenia or forming little pockets in Dipylidium. These proglottides filled with unlaid eggs are called gravid, and the release of the ova is dependent upon the dissolution of the proglottis. The male reproductive system is very similar to that of the flukes.

Morphology of the Immature Stages

Egg

The egg is a zygote surrounded by numerous yolk cells or a mature embryo contained in a protective membrane. Two types of eggs are encountered in the Class Cestoidea: the pseudophyllidean cestode has an operculate egg similar to that of certain trematodes; the cyclophyllidean tapeworm has a nonoperculate egg.

Coracidum

This is the ciliated, free-swimming larva which hatches from the egg of a pseudophyllidean tapeworm.

Onchosphere, Hexacanth Embryo, or Six-Hooked Embryo

This is the larva which hatches from the egg of a cyclophyllidean tapeworm or remains after the disappearance of the ciliated coat of the coracidum of a pseudophyllidean tapeworm.

Embryophore

The embryophore is a cellular covering which surrounds the developing onchosphere of the cyclophyllidean tapeworm. It is often erroneously called the egg shell, especially in Taenia solium and T. saginata. Some authors also consider the ciliated coat of the coracidium to be an embryophore.

Procercoid Larva

This is the larval form which develops from the hexacanth embryo. It is a spindle-shaped organism with a posterior spherical appendage (cercomer) which bears the six larval hooks. Examples, pseudophyllidean cestodes.

Plerocercoid or Sparganum Larva

A sparganum larva develops from a procercoid. It has lost the larval hooks and has developed a worm-like body with an anterior invagination. Examples, pseudophyllidean cestodes.

Cysticercoid Larva

This is a larva developed from the hexacanth embryo and characterized by the possession of an invaginated scolex and solid tail. Examples, Hymenolepis and Dipylidium.

Cysticercus Larva

The cysticercus larva is developed from the hexacanth embryo and has an invaginated scolex and a fluid-containing bladder. Example, Taenia.

Coenurus or Multiceps Larva

This larva, developed from the hexacanth embryo, possesses many invaginated scolices and a fluid-containing bladder. Example, Multiceps.

Hydatid or Echinococcus Larva

The hydatid larva is developed from the hexacanth embryo and is characterized by the possession of many invaginated scolices and many daughter bladders inside a mother bladder. Example, Echinococcus.

False Suckered Tapeworm

This term sometimes is applied to a tapeworm of the Order Pseudophyllidea.

Round Suckered Tapeworm

This term sometimes is applied to a tapeworm of the Order Cyclophyllidea.

IMMATURE STAGES OF HUMAN TAPEWORMS

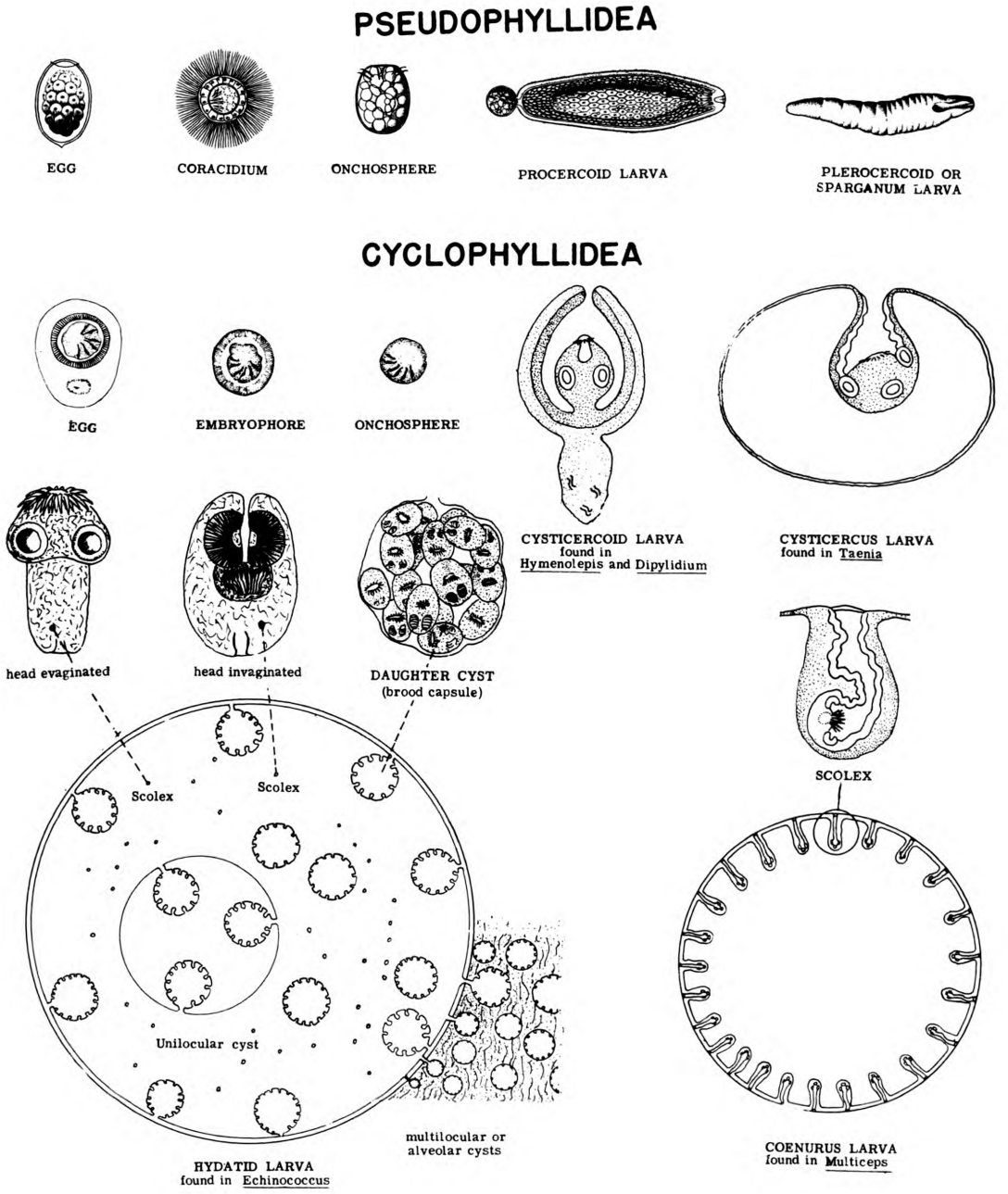


Fig. 45

Life Histories of Human Tapeworms

The life cycles of the tapeworms of man are much easier to understand if they are divided into two main groups:

Intestinal Tapeworms	Adults living in the intestinal tract of man
Somatic Tapeworms	Larvae living in the tissues of man

INTESTINAL TAPEWORMS

In this group the species represented can be placed in a series which starts with a life cycle involving one definitive and two intermediate hosts, Diphyllobothrium latum, and ends with one requiring but a single host, Hymenolepis nana (Fig. 46).

The ova of all tapeworms pass out in the feces either after being laid (Diphyllobothrium) or after being freed by the disintegration of the gravid proglottides (Taenia, Hymenolepis, Dipylidium). When the ova of Diphyllobothrium are passed, they are immature and develop upon reaching the water. The ova of Taenia, Hymenolepis, and Dipylidium are mature when passed, i.e., they contain a mature onchosphere.

The ciliated larva or coracidium which hatches from the egg of the pseudophyllidean tapeworm (Diphyllobothrium) swims in water until it is ingested by a suitable crustacean. In the gut of the crustacean it loses its ciliated envelope and is known as an onchosphere which eventually develops into a procercoid larva in the tissues of this first intermediate host. When the crustacean is eaten by an appropriate fish, the procercoid is freed in the stomach, then penetrates the intestinal wall, and enters the muscles, viscera, and connective tissues of the fish after a journey through the blood and lymph channels. It then develops into a plerocercoid or sparganum larva. Man, eating the fish raw or insufficiently cooked, digests out the sparganum which attaches itself to his intestinal wall and grows into the adult tapeworm.

The ova of the cyclophyllidean tapeworms (Taenia, Hymenolepis, Dipylidium) must be ingested in food or water by the suitable intermediate host before hatching. The hosts may be cattle, pigs, fleas or other insects, or man. In these hosts the egg hatches in the intestine, freeing the hexacanth embryo, which then penetrates the intestinal mucosa with the aid of its larval hooks and makes its way to the viscera, muscles, or connective tissue via the blood or lymph. Here it absorbs food from the surrounding tissues and matures into either a cysticercus (Taenia) or cysticercoid larva (Dipylidium, Hymenolepis). When man eats infected tissues of beef, hogs, or insects, the larva is freed, evaginates its single scolex, and, after attaching itself to the intestinal wall, grows into the mature tapeworm. In Hymenolepis nana, the larva which has used man as an intermediate host merely breaks out of the villus which housed it, evaginates the scolex, and develops into a mature worm.

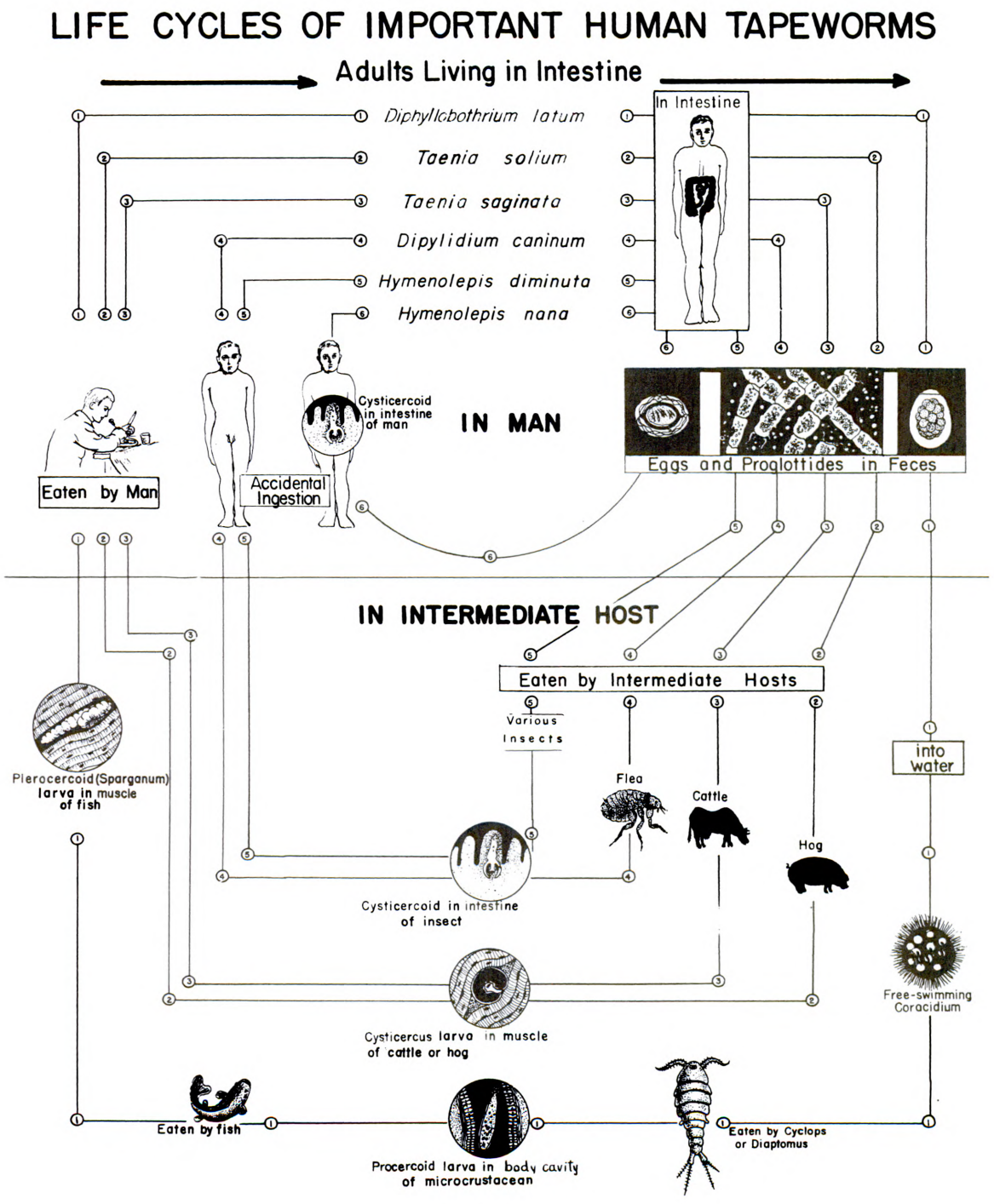

LIFE CYCLES OF IMPORTANT HUMAN TAPEWORMS
Adults Living in Intestine
Diphyllobothrium latum
Taenia solium
Taenia saginata
Dipylidium caninum
Hymenolepis diminuta
Hymenolepis nana
In Intestine
Cysticercoid in intestine of man
IN MAN
Eaten by Man
Accidental Ingestion
Eggs and Proglottides in Feces
IN INTERMEDIATE HOST
Eaten by Intermediate Hosts
Various Insects
Flea
Cattle
Hog
into water
Plerocercoid (Sparganum) larva in muscle of fish
Cysticercoid in intestine of insect
Cysticercus larva in muscle of cattle or hog
Free-swimming Coracidium
Eaten by fish
Procercoid larva in body cavity of microcrustacean
Eaten by Cyclops or Diaptomus
Naval Medical School

Fig. 46

Morphology of Adult Intestinal Tapeworms

Adult tapeworms differ greatly in their size and the number of proglottides; this is most easily presented in tabular form:

SPECIES	SIZE (in cm.)	NUMBER OF PROGLOTTIDES
Diphyllobothrium latum	300-1,000	3,000-4,000
Taenia solium	200-700	800-1,000
Taenia saginata	300-800 (occ. to 2,500)	1,000-2,000
Hymenolepis nana	2.5-5	100-200
Hymenolepis diminuta	10-60	800-1,000
Dipylidium caninum	15-70	60-175

Diphyllobothrium latum

The "broad tapeworm" or "fish tapeworm"

Geographic Distribution

This parasite is found in the lake districts of the world. It has been recorded from Italy, Switzerland, France, Bavaria, Rumania, Manchuria, Japan, Palestine, Africa, the United States (Michigan, Minnesota), Canada (Manitoba) and South America.

Morphology of Adult

The scolex is somewhat elongated and almond-shaped; it is from 2 to 3 mm. in length and 0.7 to 1.0 mm. in breadth; it is provided with dorsal and ventral bothria (suckers). The gravid proglottides usually measure from 2 to 4 mm. in length by 10 to 12 mm., or even 20 mm., in breadth. The uterus is a long coiled canal and appears as a dark rosette-shaped object in the middle of the older proglottides. The gravid proglottides frequently break off from the parent worm in chains from a few to several feet in length and are passed in the host's feces.

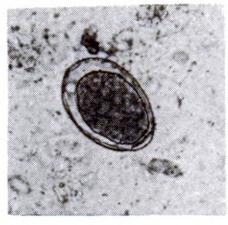


Fig. 47 - Egg of Diphyllobothrium latum

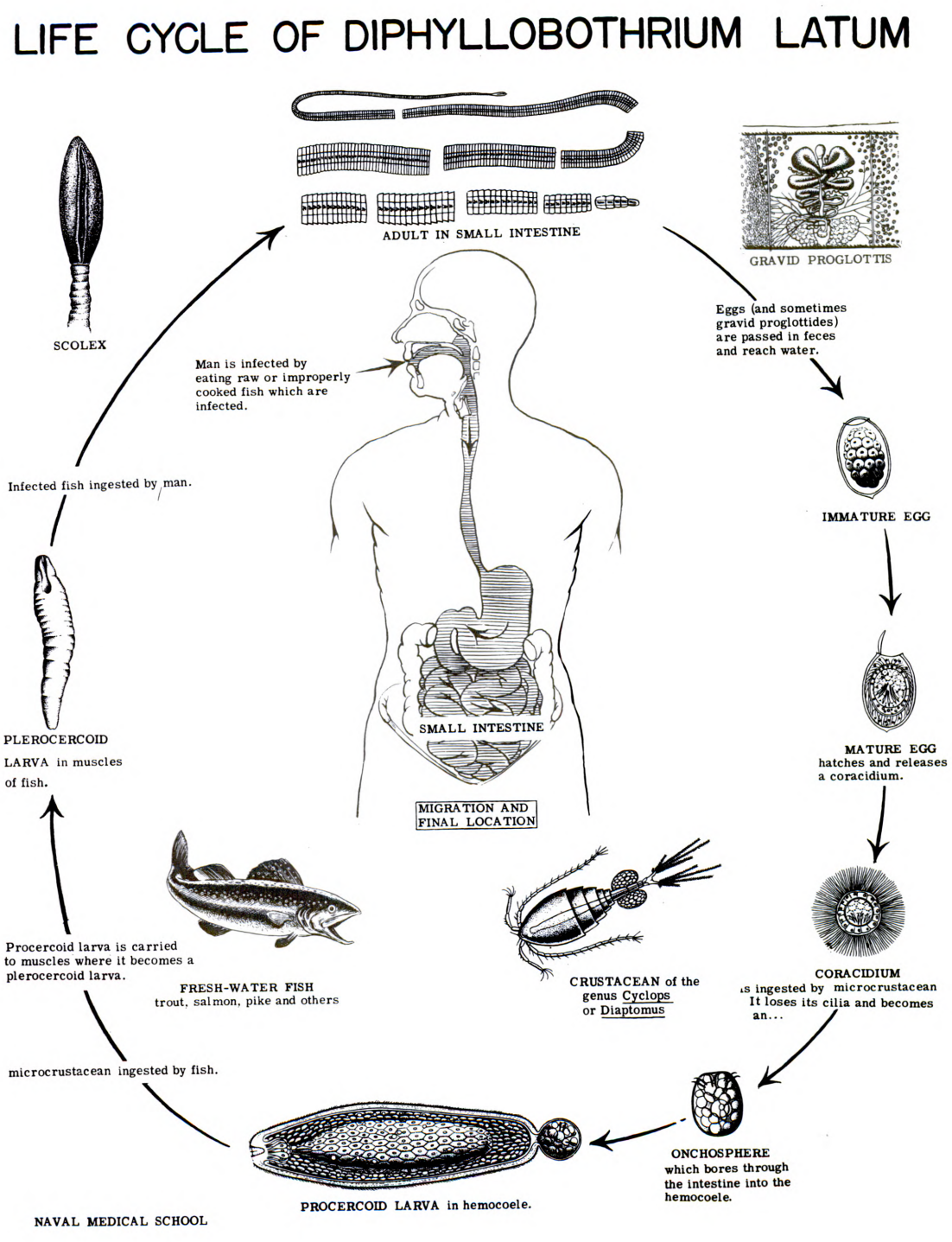

LIFE CYCLE OF DIPHYLLOBOTHRIUM LATUM
ADULT IN SMALL INTESTINE
SCOLEX
GRAVID PROGLOTTIS
Eggs (and sometimes gravid proglottides) are passed in feces and reach water.
Man is infected by eating raw or improperly cooked fish which are infected.
IMMATURE EGG
Infected fish ingested by man.
SMALL INTESTINE
MATURE EGG hatches and releases a coracidium.
PLEROCERCOID LARVA in muscles of fish.
MIGRATION AND FINAL LOCATION
CORACIDIUM
is ingested by microcrustacean It loses its cilia and becomes an...
Procercoid larva is carried to muscles where it becomes a plerocercoid larva.
FRESH-WATER FISH trout, salmon, pike and others
CRUSTACEAN of the genus Cyclops or Diaptomus
microcrustacean ingested by fish.
ONCHOSPHERE which bores through the intestine into the hemocoele.
PROCERCOID LARVA in hemocoele.
NAVAL MEDICAL SCHOOL

Fig. 48

Life Cycle (Fig. 48)

1. The adult worms are found in man, dogs, cats, bears, foxes, minks, and hogs.

2. The crustacean containing the procercoid is either ingested directly by a large carnivorous fish or by a smaller fish which is in turn eaten by the larger form.

3. The procercoid which is released in the gut of the fish penetrates the intestinal wall and makes its way by blood and lymph channels chiefly to the muscles of the fish. Here it develops into the plerocercoid or sparganum larva in from 7 to 30 days.

4. When insufficiently cooked or raw fish is eaten by man (or other definitive hosts), the plerocercoid larva attaches to the intestinal wall and grows at a rate of about 30 proglottides per day. The tapeworm becomes mature in about 1 month. The adult worm may live for as long as 20 years.

Taenia solium
The pork or "armed" tapeworm

Geographic Distribution

Cosmopolitan, but usually rare in countries in which Moslems are in the majority.

Morphology of Adult

The scolex is globular and is about 1 mm. in diameter. A rather short but distinct rostellum is present and armed with a double row of 25 to 50 hooks. The neck is fairly thin and about 5 to 10 mm. long. The gravid proglottis has a uterus which bears from five to thirteen rather thick and ramified lateral branches.

Life Cycle (Fig. 50)

Taenia saginata
The beef tapeworm

Geographic Distribution

Cosmopolitan.

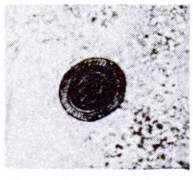


Fig. 49 - Egg of Taenia

Morphology of Adult

The scolex is pear-shaped or cubical, 1 to 2 mm. in diameter, and bears four prominent suckers which are frequently pigmented. The scolex is without a rostellum or hooks. The gravid proglottis has a uterus with 15 to 30 lateral ramifying branches.

LIFE CYCLE OF TAENIA SOLIUM

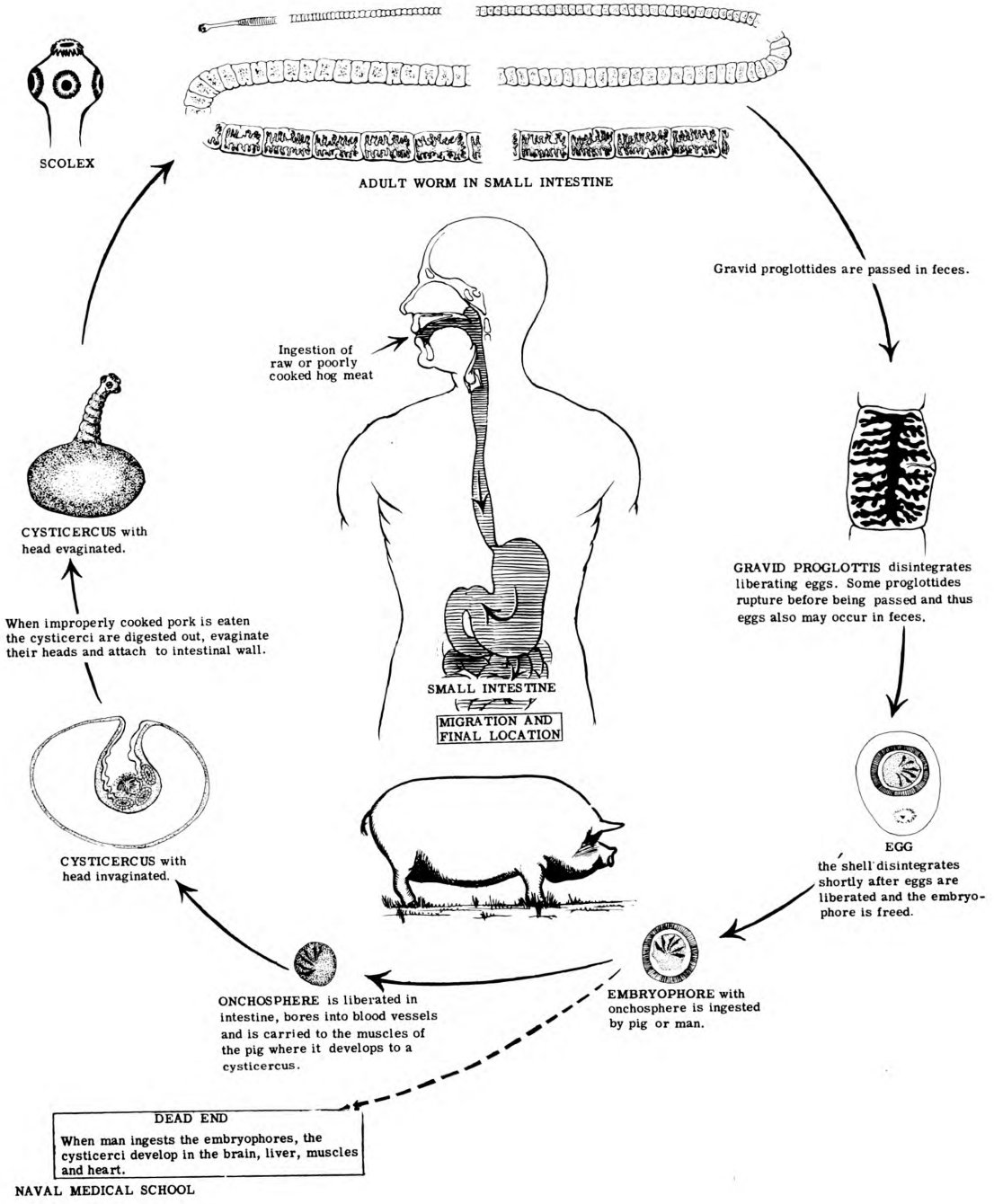


Fig. 50

Life Cycle

This is similar to T. solium (Fig. 50) with the following modification: the intermediate hosts are almost exclusively cattle. The larva is called cysticercus bovis.

Differential Characteristics of Taenia in Man

T. solium	T. saginata
Scolex with a double row of hooks	Scolex without rostellum or hooks
Length, 2 to 7 meters	Length, 3 to 5 meters
Gravid uterus, 5 to 13 branches	Gravid uterus, 15 to 30 branches
Proglottides passed in long chains	Proglottides usually passed singly
Larvae in pig (and man)	Larvae in cattle
Genital pores alternate more or less regularly from right to left margins of proglottides	Genital pores alternate irregularly from right to left margins of proglottides

Hymenolepis nana

The dwarf tapeworm

Geographic Distribution

Cosmopolitan.

Morphology of Adult

The scolex is globular, about 0.25 mm. in diameter, and the rostellum is equipped with a single row of 24 to 30 hooks. The uterus of the gravid proglottis is sac-like. The gravid proglottides are readily digested within the intestine, and the eggs occur in large numbers in the feces.

Life Cycle (Fig. 52)

1. The definitive hosts are man, mice, and rats. No intermediate host is necessary although various insects may serve as transmitters under experimental conditions.
2. The gravid proglottides often rupture within the intestine, liberating the eggs which are mature when passed. Each gravid proglottis contains from 80 to 180 eggs.

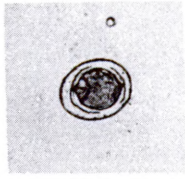
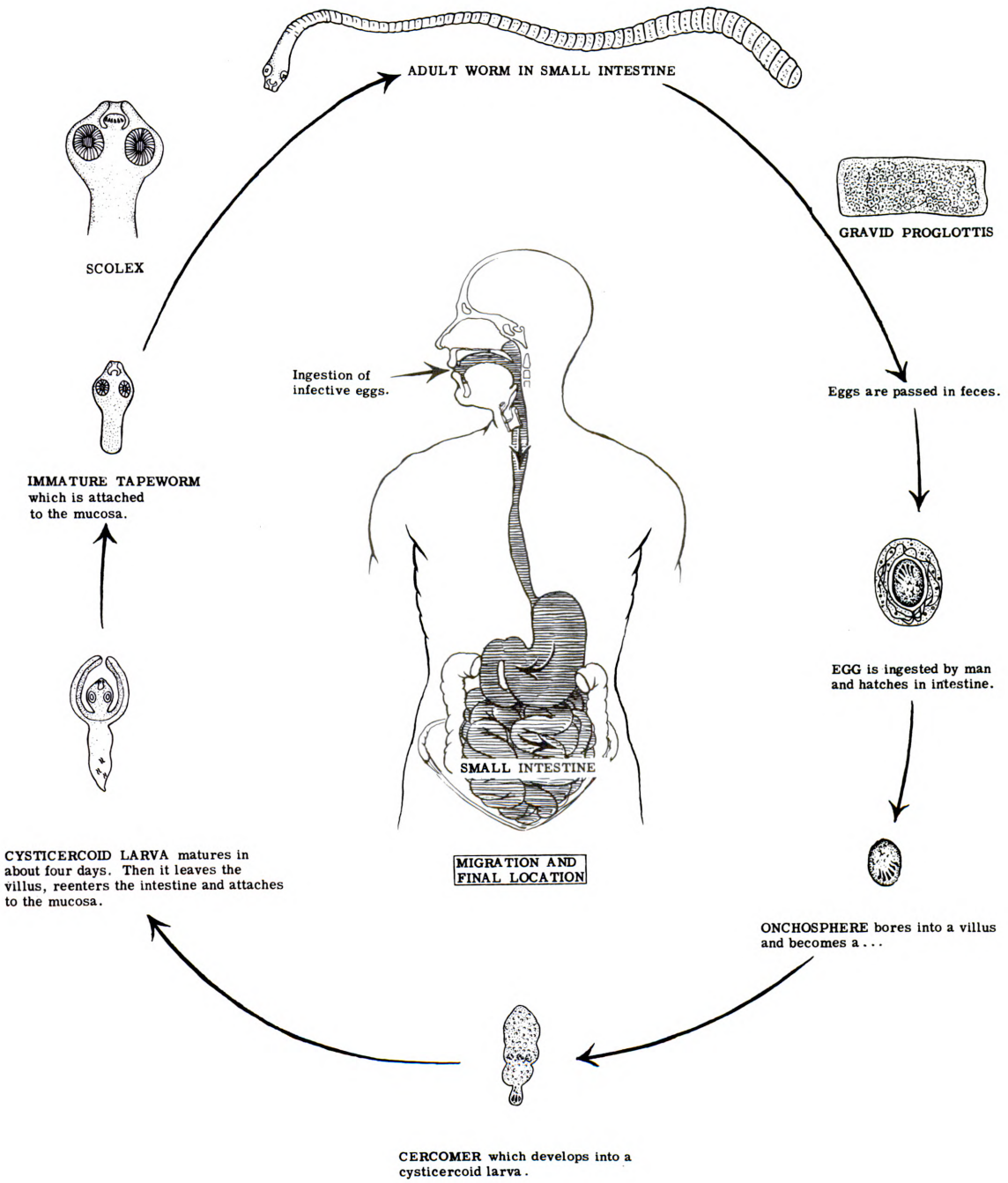


Fig. 51 - Egg of Hymenolepis nana

LIFE CYCLE OF HYMENOLEPIS NANA



NAVAL MEDICAL SCHOOL

Fig. 52

3. The eggs are immediately infective for the same or another host. When swallowed, the egg hatches and the onchosphere penetrates a villus and becomes a cysticercoid larva within 90 hours.

4. The cysticercoid larvae break out of the villi and attach to the intestinal mucosa, becoming adult worms in 10 to 12 days.

5. It is possible that hyperinfection may take place. In this case, the egg liberated in the intestine immediately hatches, and the onchosphere penetrates the intestinal wall. This phenomenon has been observed in mice experimentally, but it is not proven for man.

6. Some workers have found that fleas and certain beetles may act as hosts for the cysticercoids and that ingestion of such insects by a definitive host results in the development of the adult stage.

Hymenolepis diminuta
The mouse tapeworm

Geographic Distribution

Cosmopolitan.

Morphology of Adult

The scolex is very small and club-shaped. The four suckers are small, and at the apex of the scolex there is a rudimentary unarmed rostellum. The arrangement of the reproductive organs in each proglottis is similar to that for H. nana.

Life Cycle (Fig. 46)

1. The definitive hosts are rats and mice. About 100 cases of infection by this worm have been reported in man. The majority of these infections occurred in children under 3 years of age.

2. An intermediate host is always required for the completion of the life cycle. Suitable intermediate hosts are larvae and adults of meal moths, nymphs and adults of earwigs, adults of various grain beetles, the larvae of fleas, and myriapods. Human infection results from eating such foods as dried fruits, precooked breakfast cereals, and foods which are eaten unheated and in which the grain insects, infected from rat or mouse droppings, are present.

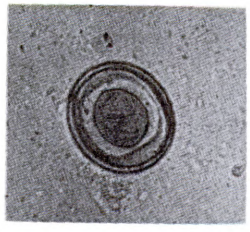


Fig. 53 - Egg of Hymenolepis diminuta

Dipylidium caninum

The dog tapeworm

Geographic Distribution

Cosmopolitan.

Morphology of Adult

The scolex is small and rhomboidal. The rostellum is armed with about 60 hooks arranged in three or four circles. The gravid proglottides are urn- or vase-shaped. Each proglottis has a double set of genital organs, and the two genital pores in each proglottis are bilateral in position.

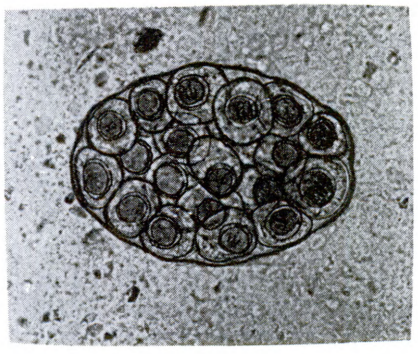


Fig. 54 - Cluster of eggs of Dipylidium caninum

Life Cycle (Fig. 46)

1. Dogs, cats, and occasionally children are the hosts of the adult tapeworms. The eggs are ingested by larval fleas, and the onchospheres bore through the intestinal wall into the hemocoele where they develop into cysticercoid larvae.

LABORATORY DIAGNOSIS OF INTESTINAL TAPEWORM INFECTIONS

The laboratory diagnosis of the intestinal tapeworms is made by finding and identifying the characteristic ova or proglottides in the feces; occasionally scolices may be recovered and identified. The only certain criterion of cure is the finding of the scolex of the worm; if the head is not removed, the worm may regenerate. The hunt is often laborious and difficult. Methods of finding the ova or embryophores should include the direct smear, sedimentation, and centrifugation. Flotation technics are not satisfactory for the operculate ova of Diphyllobothrium or for the embryophores of Taenia saginata and T. solium; they give, however, excellent results with Hymenolepis nana and H. diminuta. Russian workers and others have recently shown a substantial increase in positive diagnoses of Taenia saginata by scraping the perianal skin. The NIH swab and the scotch tape technic should both be applicable; such methods may be of value for T. solium.

Where gravid proglottides are passed unbroken in the feces (Dipylidium, Taenia), screening methods must be used. A saline purge will usually cause some of the segments to break away and they may then be recovered; this procedure may be followed when the only proglottides passed are degenerate and not identifiable. The

DIFFERENTIAL CHARACTERS OF CESTODE EGGS

SPECIES	SIZE (in microns)	SPECIAL FEATURES
Diphyllobothrium latum	55-75 x 40-55; average 70 x 45	Indistinctly operculate with knob-like thickening on abopercular end; immature
Taenia solium	30-40 (diameter); average 35	Nonoperculate; embryo with six hooks; thick, brown, radially striated embryophore
Taenia saginata	30-40 x 20-30; average 35 x 25	
Hymenolepis nana	30-60 (diameter); average 47 x 37	Nonoperculate; embryo with six hooks; poles of inner shell with filaments
Hymenolepis diminuta	70-85 x 60-80; average 72 x 65	Nonoperculate; embryo with six hooks; poles of inner shell without filaments
Dipylidium caninum	20-50 (diameter); average 40 x 36	Nonoperculate; embryo with six hooks; usually in packets of 10-12 ova

DIFFERENTIAL CHARACTERS OF GRAVID PROGLOTTIDES

SPECIES	SIZE (in mm.)	SPECIAL FEATURES
Diphyllobothrium latum	2-4 x 10-12; "broader than long"	Rosette-shaped, coiled uterus; no lateral gonopore
Taenia saginata	16-20 x 5-7; "longer than broad"	15 or more main uterine branches; single lateral gonopore
Taenia solium	10-12 x 5-7; "longer than broad"	13 or less main lateral uterine branches; single lateral gonopore
Hymenolepis nana	0.15-0.3 x 0.8-0.9; "broader than long"	Uterus irregular, sac-like; single lateral gonopore
Hymenolepis diminuta	0.75 x 2.5; "broader than long"	Uterus irregular, sac-like; single lateral gonopore
Dipylidium caninum	10-12 x 2.5-3.0; "longer than broad"	Two gonopores on each segment; uterus with polygonal egg sacs

gravid proglottides and other sections of the adult worm may be identified by the key (page 184). Thirteen or less main uterine branches on each side identifies T. solium, fifteen or more T. saginata; the pinkish gravid proglottis with two gonopores and containing a large number of egg sacs is characteristic of Dipylidium.

Man's immunological response to the intestinal tapeworms is generally slight, and serological and allied tests have not proven satisfactory.

SOMATIC TAPEWORMS

The life cycles of these worms, the larvae of which live in the tissues of man, are shown (page 134). Hymenolepis nana should be included in this group, but since its larval stage is of very little importance, it has been omitted. It must be realized that all tapeworms, except H. nana, must have an intermediate host for the larval stages which can be ingested by the definitive host. The customs of the human race have made it almost impossible for man to act as functional intermediate host for any tapeworm. As a result, those tapeworms which happen to get into man are in a blind alley and are unable to continue their life cycles (except under most unusual circumstances).

The invasion of the human body by larvae has been observed with four species of tapeworms. One species is a member of the Order Pseudophyllidea and the other three of the Order Cyclophyllidea. The life cycle of the pseudophyllidean involved is similar to that already described for Diphyllobothrium latum. The condition produced is known as sparganosis.

Infection by the larvae of the cyclophyllidean tapeworms is dependent upon the ingestion of the egg by man in food or water. Hatching occurs in the small intestine, the onchosphere penetrates the intestinal wall and passes to the body tissues, and the larvae develop. The larvae of three tapeworms, Taenia solium, Multiceps multiceps, and Echinococcus granulosus, form an interesting series showing increased complexity. Thus the cysticercus larva of Taenia solium has one invaginated scolex and one bladder; the coenurus larva of Multiceps multiceps has many invaginated scolices and one bladder; and the hydatid larva of Echinococcus granulosus has many invaginated scolices and many bladders. Therefore, one adult tapeworm may develop from each cysticercus, hundreds from each coenurus, and thousands from each hydatid. Invasion of the body by the larvae of Taenia solium is called cysticercosis; by M. multiceps, coenurosis; and by E. granulosus, echinococcosis.

Cysticercosis and echinococcosis are not uncommon in man, while sparganosis and coenurosis are very rare.

Echinococcosis or Hydatid Disease Caused by Echinococcus granulosus

This is the most important of the four infections caused by the larval tapeworms. It is common in sheep-raising countries, such as Australia, New Zealand, South

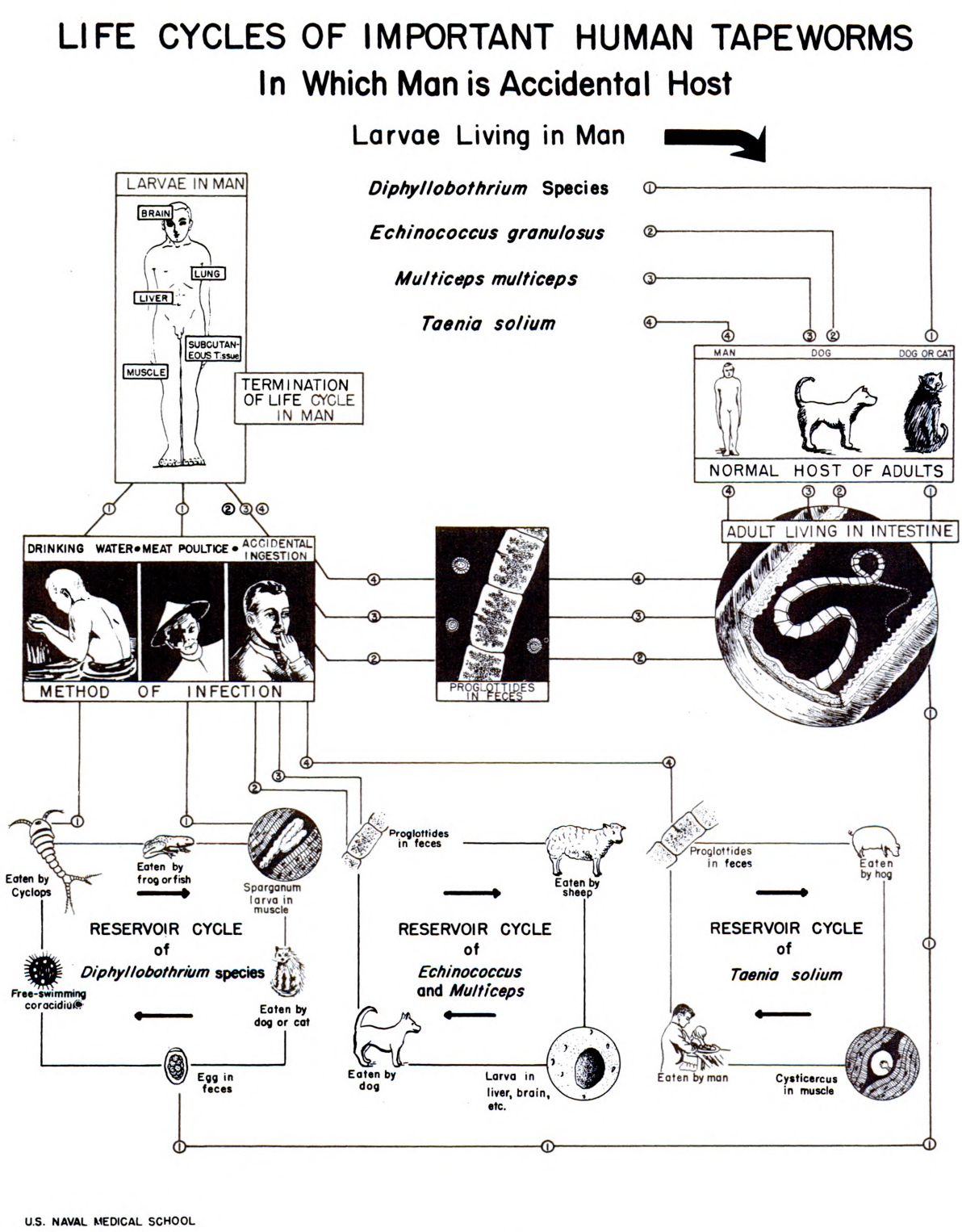

LIFE CYCLES OF IMPORTANT HUMAN TAPEWORMS
In Which Man is Accidental Host
Larvae Living in Man
LARVAE IN MAN
BRAIN
LUNG
LIVER
SUBCUTAN-EOUS Tissue
MUSCLE
TERMINATION OF LIFE CYCLE IN MAN
Diphyllobothrium Species
Echinococcus granulosus
Multiceps multiceps
Taenia solium
MAN
DOG
DOG OR CAT
NORMAL HOST OF ADULTS
ADULT LIVING IN INTESTINE
DRINKING WATER • MEAT POULTICE • ACCIDENTAL INGESTION
METHOD OF INFECTION
PROGLOTTIDES IN FECES
Eaten by Cyclops
Eaten by frog or fish
Sparganum larva in muscle
Free-swimming coracidium
Eaten by dog or cat
Egg in feces
RESERVOIR CYCLE of Diphyllobothrium species
Proglottides in feces
Eaten by sheep
Eaten by dog
Larva in liver, brain, etc.
RESERVOIR CYCLE of Echinococcus and Multiceps
Proglottides in feces
Eaten by hog
Eaten by man
Cysticercus in muscle
RESERVOIR CYCLE of Taenia solium
U.S. NAVAL MEDICAL SCHOOL

Fig. 55

America, Africa, Iceland, and Japan. Autochthonous infections in the United States are infrequent. The normal host of the adult worm (Fig. 57) is the dog, whereas sheep and cattle are the most common larval hosts. Human infections result from the accidental ingestion of the ova from the feces of dog. The ova hatch in the intestine, and the onchosphere penetrates the wall, enters the lymph or blood, and is carried to various parts of the body. There the hydatid larva develops (Fig. 45). The damage produced by a simple or unilocular hydatid is largely the result of pressure on surrounding organs, hence the seriousness of the infection depends upon the organ infected and the size of the cyst. In addition, "alveolar" or multilocular growths may develop; these may break off from the parent cyst and be carried through blood and lymph vessels to other parts of the body. These secondary echinococcoses usually give rise to grave consequences.

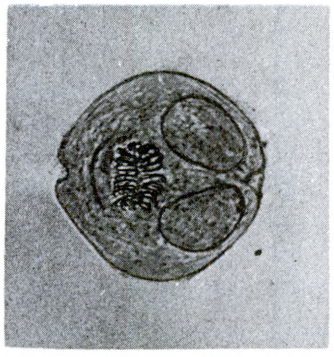


Fig. 56 - Single scolex of Echinococcus granulosus

Cysticercosis Cause by Taenia solium

Man becomes infected with the larval form known as Cysticercus cellulosae through the ingestion of the eggs of T. solium. The cysticerci may develop in any organ. The frequency of their occurrence as determined by autopsies is given in the following order: brain, eye, muscles, heart, liver, lungs, and abdominal cavity. The gravity of the infection depends upon the organ involved and the number of larvae which have invaded the tissues. Autoinfection is very common, and because of this fact it is of vital importance that every case of T. solium infection be treated and the patient be made aware of the dangers of accidental ingestion of the eggs. Details of the life cycle have already been given (Figs. 45 and 55). Man apparently does not become infected with Cysticercus bovis, the larval stage of Taenia saginata.

Sparganosis Caused by Diphyllobothrium Species

Man is infected in one of two ways: (1) By drinking water containing Cyclops infected with the procercoid larvae; (2) By using frog muscle containing the sparganum or plerocercoid larva as a poultice for lacerated tissue. Cases have been reported in Japan, China, and the United States. Details of structure and life cycle have been previously shown (Figs. 45 and 55).

Coenurosis Caused by Multiceps multiceps

The normal larval hosts of this worm are sheep and cattle. Man is infected by ingesting the eggs from the feces of dogs, the definitive host. In sheep and cattle the larvae live in the brain. The larval stage in man (Fig. 45) may be found in either connective or nervous tissue.

LIFE CYCLE OF ECHINOCOCCUS GRANULOSUS

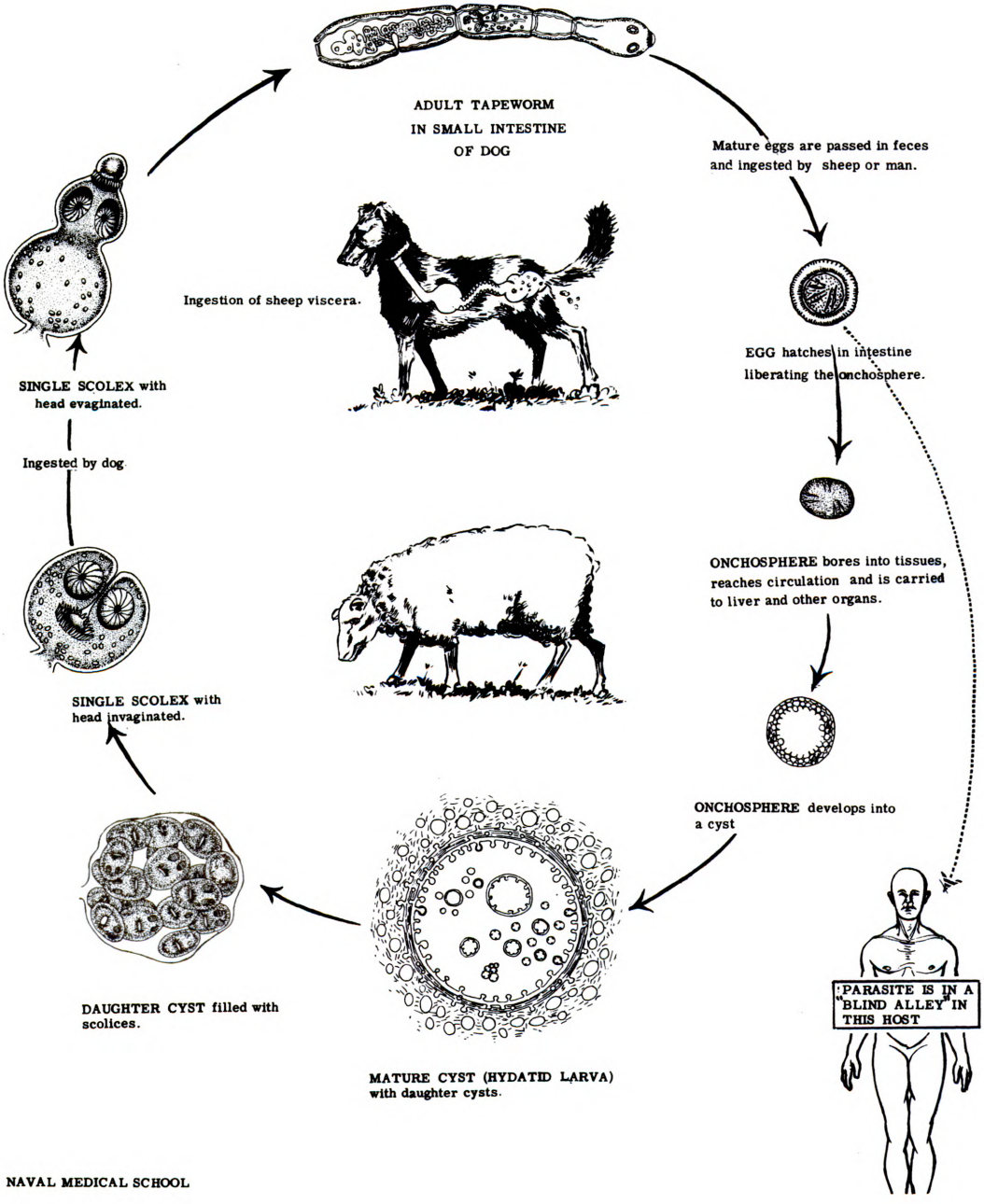


Fig. 57

LABORATORY DIAGNOSIS OF SOMATIC TAPEWORM INFECTIONS

Serologic and allied tests offer the most practical method of diagnosis. Since the somatic forms cause profound tissue damage and produce a powerful immune response, such tests are much more important in these diseases than in the intestinal infections. The reactions are of a group character and must be carefully evaluated. In some cases a negative test does not exclude infection.

The most delicate and specific test for echinococcosis is the intradermal or Casoni reaction. Complement fixation and precipitation tests are also available but are of less value except in old or complicated cases. Similar methods are used with somewhat less reliable results in the diagnosis of cysticercosis. Sparganosis has been successfully diagnosed in monkeys and in a few human cases by serologic tests.

In cases where a lung cyst has suppurated or a liver cyst has ruptured into a lung, fragments and hooklets may be found in the sputum. Exploratory aspiration is dangerous; if leakage of fluid occurs or if the cyst ruptures, a violent reaction follows, anaphylactoid in type. The scattered scolices tend to become implanted and give rise to new cysts. Again the diagnosis is made from the identification of free scolices or of scattered hooklets from those that have disintegrated.

The cysticercus larvae may be diagnosed after biopsy or at autopsy by the typical miniature scolex of Taenia solium. Very often it is impossible to excise the encysted larvae because of their location in the body. Roentgen ray examination is of value, especially where calcification has occurred. Hydatid cysts of the lung are characterized by their sharp outline.

The roundworms (nematodes) of man may be easily distinguished from all other helminths by the following characters: (1) body thread-like and cylindrical (round in cross section); (2) unsegmented; (3) sexes separate; (4) a complete digestive tract with mouth and anus.

Morphology

Method of Attachment

The nematodes which live in the tissues of man require no attachment organs, but forms which inhabit the intestine maintain their position in one of three ways: (1) by their own movements (Ascaris, Enterobius); (2) by mouth structures (Necator, Ancylostoma); (3) by actual penetration of the intestinal mucosa. The entire worm may enter the intestinal wall (Trichinella, Strongyloides) or only the anterior portion of the body may be inserted (Trichuris).

Integument and Body Wall

The entire body of a roundworm is covered by a hyaline, noncellular, homogeneous, and impervious layer, the cuticle, which is secreted by the hypodermis. The musculature consists of a single layer of longitudinal fibers.

Nervous System

This system consists of six longitudinal nerves connected by a ring commissure near the anterior end. Special sense organs, touch and chemoreceptors, are located at the anterior and posterior ends of the worm.

Digestive System

The mouth of the roundworm opens into a buccal capsule which in turn leads into a muscular esophagus. The latter is continuous with a long, thread-like intestine which dilates to form a rectum, emptying near the posterior end through the anus. The buccal capsule and esophagus are lined by a continuation of the cuticle which forms teeth and other structures in some species.

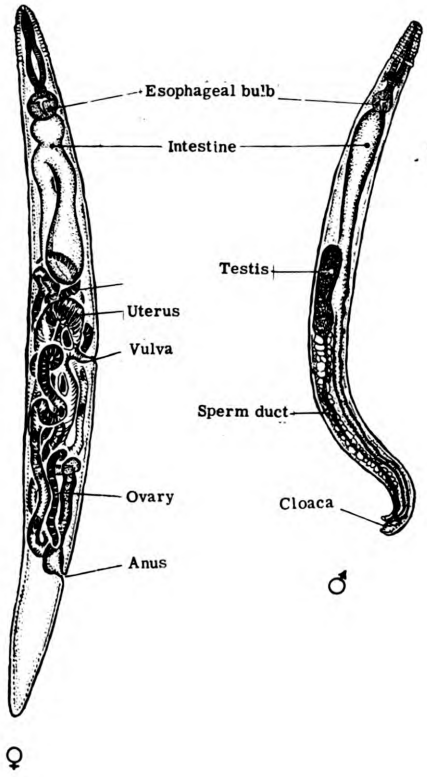


Fig. 58 - A nematode

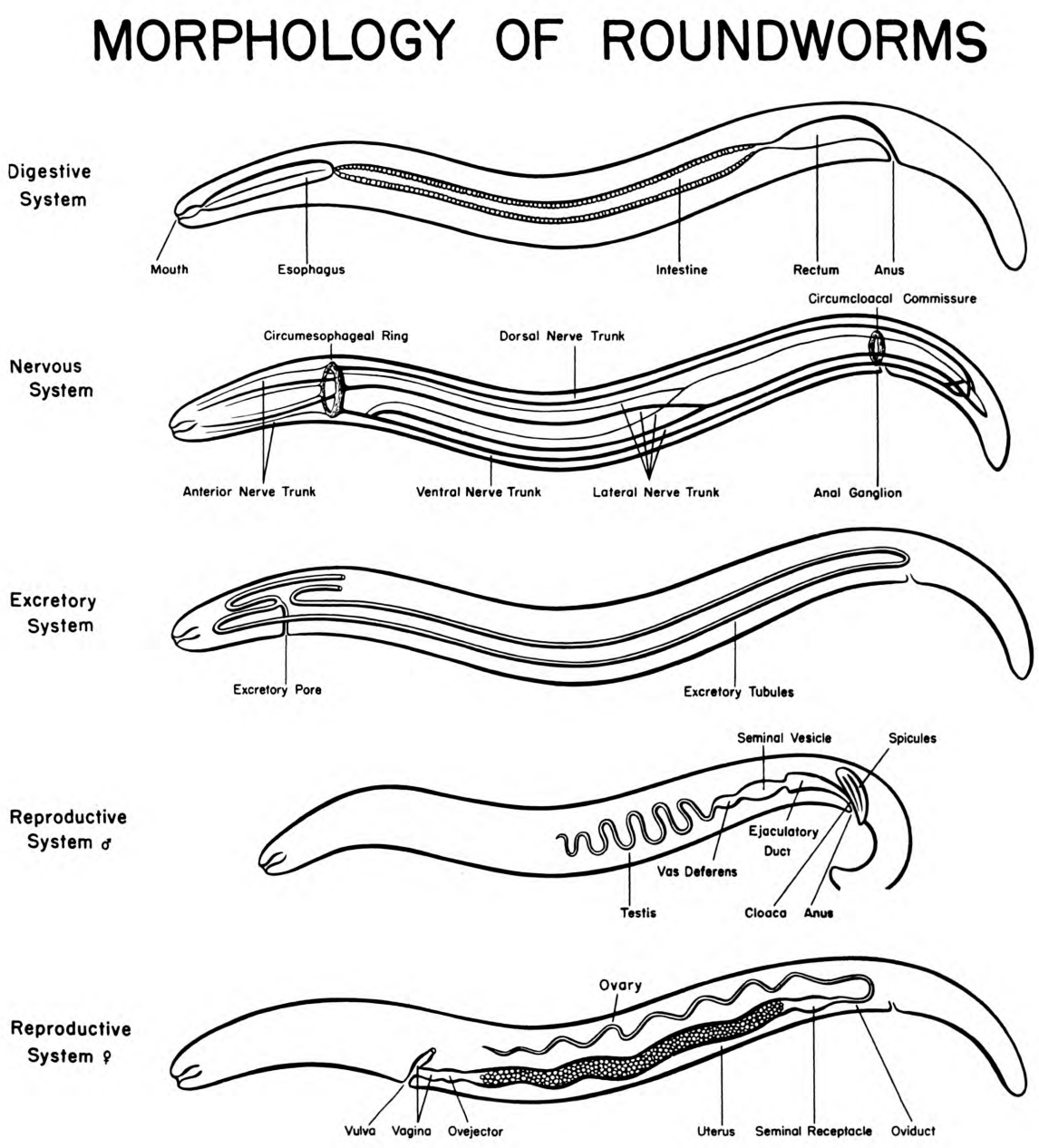

MORPHOLOGY OF ROUNDWORMS
Digestive System
Mouth
Esophagus
Intestine
Rectum
Anus
Nervous System
Circumcloacal Commissure
Circumesophageal Ring
Dorsal Nerve Trunk
Anterior Nerve Trunk
Ventral Nerve Trunk
Lateral Nerve Trunk
Anal Ganglion
Excretory System
Excretory Pore
Excretory Tubules
Reproductive System ♂
Seminal Vesicle
Spicules
Ejaculatory Duct
Vas Deferens
Testis
Cloaca
Anus
Reproductive System ♀
Ovary
Vulva
Vagina
Ovejector
Uterus
Seminal Receptacle
Oviduct

Fig. 59

Excretory System

This system typically consists of two lateral, longitudinal tubules opening to the exterior by a ventral pore just posterior to the esophagus. It varies greatly, however, in different species.

Reproductive Systems

The male system consists of a single highly convoluted tubule leading posteriorly and differentiated into testis, vas deferens, seminal vesicle, and ejaculatory duct. The latter opens, along with the intestine, into a common chamber called the cloaca. The male worm is smaller than the female and often has its posterior end more or less modified. The posterior end is expanded in the hookworms to form a copulatory bursa, and the tail of the male is curved ventrally in most other species. The accessory sexual apparatus in the male nematode may be highly developed, with one or two spicules which lie in a sheath dorsal to the cloaca and ejaculatory duct. In addition, there may be another small, accessory chitinous structure called the gubernaculum associated with the sexual organs. The female system consists of one or two tubules differentiated into ovary, oviduct, seminal receptacle, uterus, and vagina. The opening of the female reproductive system is separate from that of the digestive system and may be located far anteriorly.

Life Histories of Human Roundworms

The roundworms pass through a series of molts and increase in size during their life cycles. On the basis of their habitat in the human body, they are divided into the intestinal roundworms (Strongyloides, Necator, Ancylostoma, Ascaris, Trichuris, and Enterobius) and the somatic or tissue roundworms (Wuchereria, Loa, Onchocerca, Acanthocheilonema, Mansonella, and Dracunculus).

The life cycle of Trichinella is different from that of other roundworms in that the same animal is always the definitive as well as intermediate host. It is considered separately for this reason.

INTESTINAL ROUNDWORMS

The life cycles of these roundworms may be considered as a series, each succeeding species being a more highly developed parasite. In the order of increasing adaptation to parasitism, the series goes from Strongyloides through Necator, Ancylostoma, Ascaris, and Trichuris to Enterobius. Inasmuch as none of these intestinal forms requires an intermediate host, the only differences in cycles are: (1) the stages and period of time spent outside man and (2) the larval migrations within man. There are two larval stages which are defined as follows: rhabditiform larva, the first larval stage, which is a feeding, noninfective form; filariform larva, the second larval stage, which is the nonfeeding, infective form.

The most primitive form, Strongyloides, is able to maintain itself as a free-living form. The parasitic cycle is initiated when the rhabditiform larva changes into a filariform stage, penetrates the skin of man, and is carried by the blood to the lungs. There it breaks out of the capillaries into the air spaces, passes up the trachea to the pharynx, and moves down the esophagus to reach the stomach and small intestine.

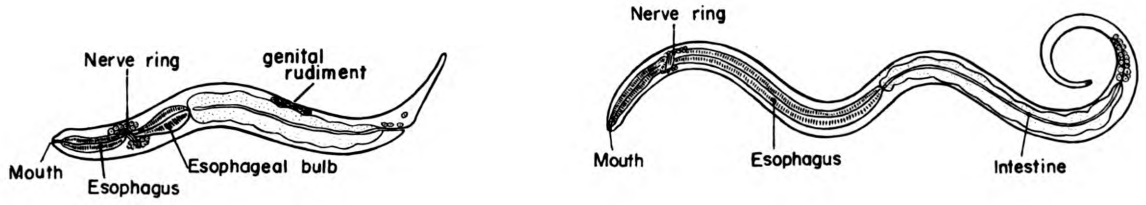


Fig. 60 - Rhabditiform and filariform larvae of intestinal roundworms

The second forms, Necator and Ancylostoma, have no free-living adult stages, although the egg and the two larval stages live outside the host. A larval migration similar to that of Strongyloides occurs after the filariform larva penetrates the skin.

Ascaris is an example of the third step in the series. Only the egg lives outside man, and it requires about 2 weeks in warm, moist soil before it becomes mature and infective. The egg must be ingested by man and hatches in the intestine. The larva penetrates the mucosa and migrates in the same manner as Necator and Strongyloides.

The fourth form is Trichuris which is similar to Ascaris except that the larva, escaping from the egg in the intestine, attaches itself to the mucosa and grows to the adult stage without the larval migration.

The last example is Enterobius in which the egg is mature and infective when passed by the host. The adult develops directly from the larva which escapes from the ingested egg.

Strongyloides stercoralis

Geographic Distribution

This worm, like the hookworm, is dependent on a warm, moist soil for completion of its life cycle. Its distribution, in general, parallels that of human hookworms.

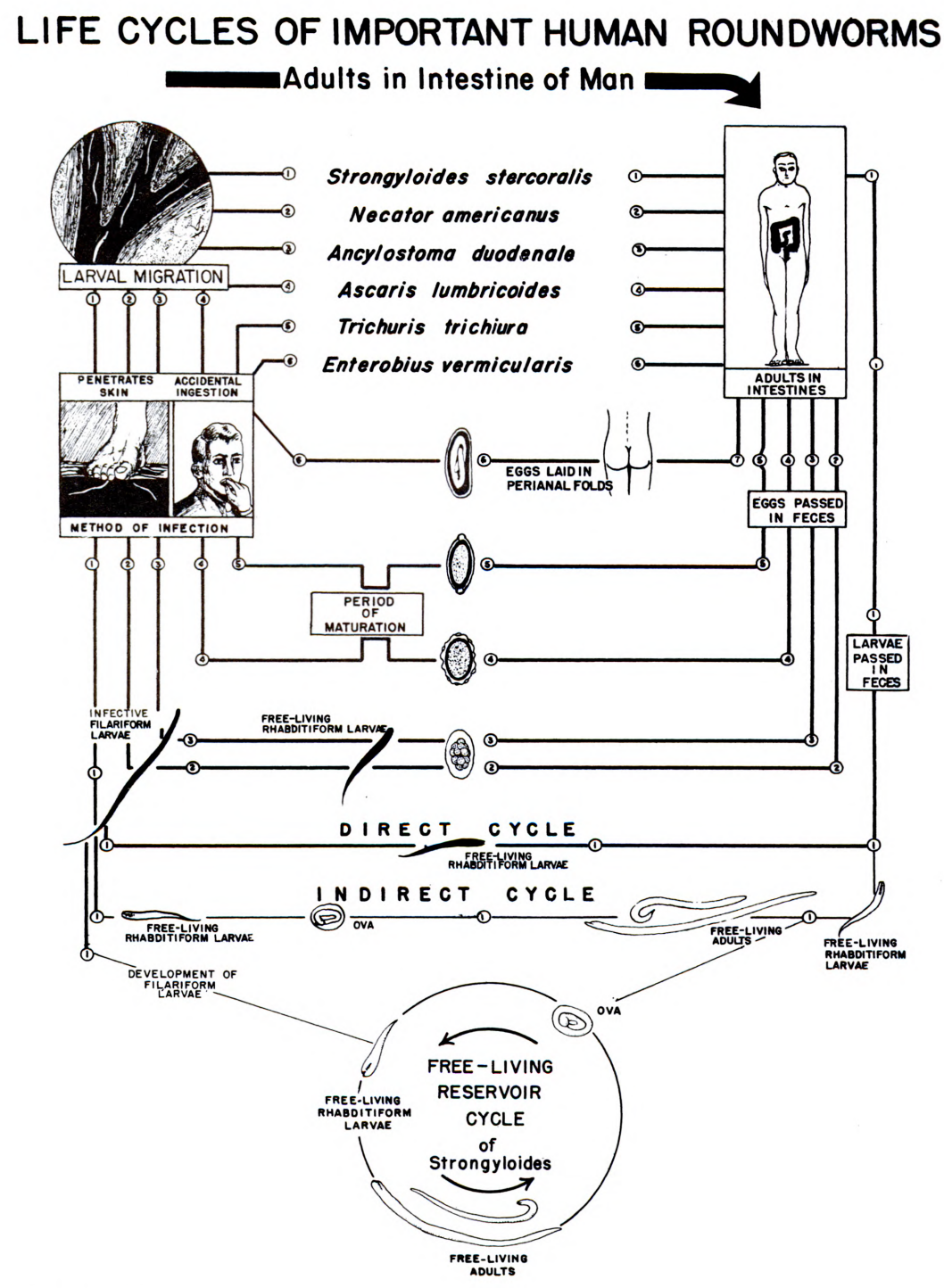

LIFE CYCLES OF IMPORTANT HUMAN ROUNDWORMS
Adults in Intestine of Man
Strongyloides stercoralis
Necator americanus
Ancylostoma duodenale
Ascaris lumbricoides
Trichuris trichiura
Enterobius vermicularis
LARVAL MIGRATION
PENETRATES SKIN
ACCIDENTAL INGESTION
METHOD OF INFECTION
ADULTS IN INTESTINES
EGGS LAID IN PERIANAL FOLDS
EGGS PASSED IN FECES
PERIOD OF MATURATION
LARVAE PASSED IN FECES
INFECTIVE FILARIFORM LARVAE
FREE-LIVING RHABDITIFORM LARVAE
DIRECT CYCLE
FREE-LIVING RHABDITIFORM LARVAE
INDIRECT CYCLE
FREE-LIVING RHABDITIFORM LARVAE
OVA
FREE-LIVING ADULTS
FREE-LIVING RHABDITIFORM LARVAE
DEVELOPMENT OF FILARIFORM LARVAE
OVA
FREE-LIVING RESERVOIR CYCLE of Strongyloides
FREE-LIVING RHABDITIFORM LARVAE
FREE-LIVING ADULTS
Naval Medical School

Fig. 61

Morphology

Adults - The free-living and the parasitic males are practically identical, being about 0.7 mm. long, fusiform in shape, and having a pointed, curved tail. Associated with the reproductive organs are two spicules and a gubernaculum. The esophagus has a posterior bulb as in the rhabditiform larva. The females differ somewhat in the free-living and parasitic generations. The free-living female is about 1 mm. long and rather stout. The uterus has two arms, one extending anteriorly and one posteriorly from the vulva which opens ventrally at approximately the middle of the body. There is an esophageal bulb similar to that in the male. The parasitic female is longer, 2.2 mm., and more slender. The vulva is slightly posterior to the middle of the body. The esophagus is much lengthened and without a bulb.

Eggs - The eggs resemble hookworm eggs, being about the same size, 50 microns, and having a similar thin, transparent shell. They are very rarely seen.

Rhabditiform larva - When first hatched, the rhabditiform larva measures about 0.25 mm., but by the time it is passed in the feces it may have grown to twice that size. Like the rhabditiform larva of hookworms, it has a pyriform, posterior esophageal bulb, but it differs in having a much shorter and broader buccal capsule.

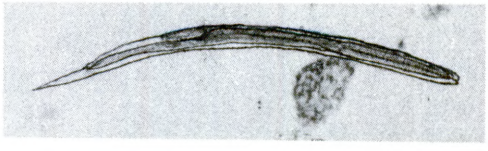


Fig. 62 - Rhabditiform larva of Strongyloides stercoralis

Filariform larva - This is the infective larva which penetrates the skin. It is about 0.55 mm. in length and can be distinguished from the infective larva of hookworms by its notched tail.

Life Cycle (Fig. 63)

The general plan of the life cycle of S. stercoralis is like that of the hookworms: the filariform infective larva develops in the soil, penetrates the skin, and migrates to the intestines via the heart and lungs. Eggs are laid and hatch in the intestinal mucosa of man; thus, rhabditiform larvae, not eggs, are passed in the feces. This parasite is capable of a free-living, continuous existence in the soil, males and females producing eggs from which the rhabditiform larvae hatch and develop directly into adults. These again produce eggs and the cycle continues, generation after generation, if conditions of the soil are favorable. When unfavorable conditions arise, the rhabditiform larvae may develop into filariform larvae instead of adults and, should these succeed in making contact with man, parasitic generations develop. In the parasitic generations, the rhabditiform larvae may metamorphose into filariform larvae without leaving the intestines of the host, thus giving rise to internal reinfection (hyperinfection).

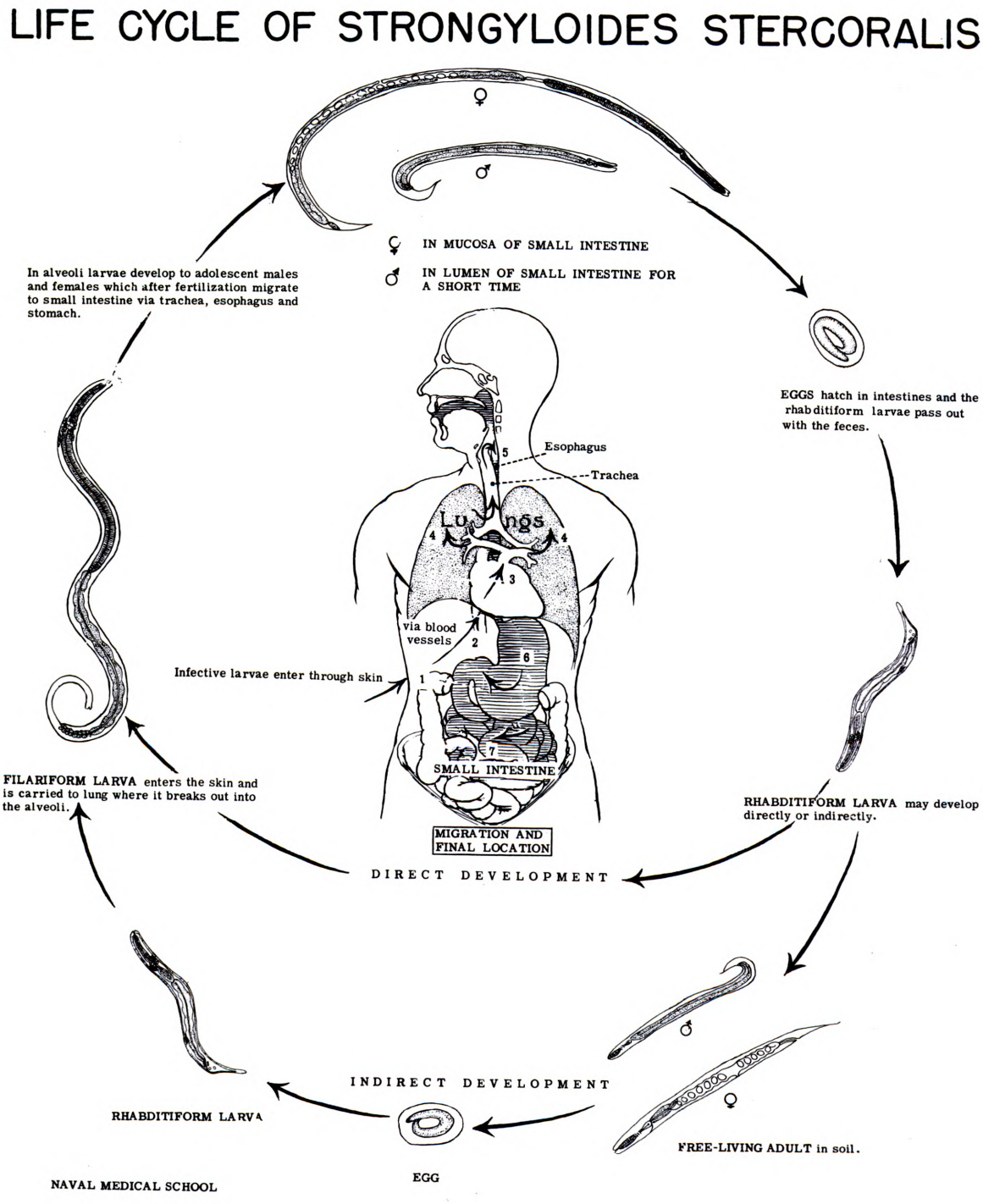

LIFE CYCLE OF STRONGYLOIDES STERCORALIS
♀ IN MUCOSA OF SMALL INTESTINE
♂ IN LUMEN OF SMALL INTESTINE FOR A SHORT TIME
In alveoli larvae develop to adolescent males and females which after fertilization migrate to small intestine via trachea, esophagus and stomach.
EGGS hatch in intestines and the rhabditiform larvae pass out with the feces.
Esophagus
Trachea
Lungs
via blood vessels
Infective larvae enter through skin
SMALL INTESTINE
MIGRATION AND FINAL LOCATION
RHABDITIFORM LARVA may develop directly or indirectly.
DIRECT DEVELOPMENT
FILARIFORM LARVA enters the skin and is carried to lung where it breaks out into the alveoli.
INDIRECT DEVELOPMENT
RHABDITIFORM LARVA
FREE-LIVING ADULT in soil.
EGG
NAVAL MEDICAL SCHOOL

Fig. 63

Hookworms

There are two species of hookworms of major importance to man, Ancylostoma duodenale (Old World) and Necator americanus (New World).

Geographic Distribution

A. duodenale is found principally in southern Europe, northern Africa, China, and Japan; N. americanus is found in southern United States, Central America, the Caribbean Islands, and the northern parts of South America as well as in Central and South Africa, southern Asia, and Polynesia.

Morphology

Some of the important structures used to differentiate the two species of hookworms are listed below:

	A. duodenale	N. americanus
Approximate size, mm.	Male, 10; female, 12	Male, 8; female, 10
Position of head	Head continues in same curvature as that of body	Head turned dorsally and at an angle with general curvature of body, giving hooked appearance to anterior end
Buccal capsule	Two pairs of ventral, claw-like teeth and one pair of dorsal, knob-like teeth	Two ventral cutting plates and two rudimentary, dorsal plates
Copulatory bursa	Dorsal ray divides in the distal third, each division ending in three digitations (tripartite)	Dorsal ray divides at the base, each division ending in two digitations (bipartite)
Copulatory spicules	Two hair-like spicules	Similar but fused and barbed at the tip
Vulva	In the posterior half	In the anterior half
Spine at posterior tip of female	Present	Absent
Egg size (microns)	34-40 x 56-60	36-40 x 64-76
Filariform larva	Larger, flatter head; esophagus narrower and without constriction	Smaller, more cylindrical head; esophagus broader with definite constriction

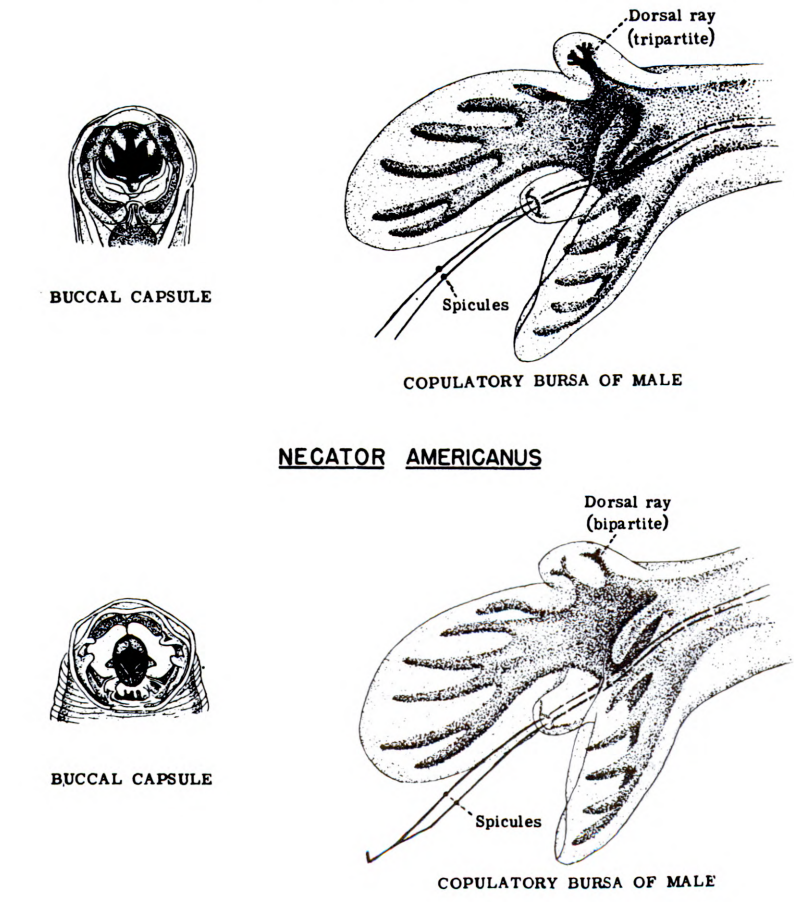


Fig. 64 - Characters for differentiating important hookworms of man

Life Cycles (Fig. 66)

The life cycles of the two important species of hookworms of man are practically the same. The adults fix themselves by their buccal capsules to the mucosa of the small intestine and feed on blood and lymph from their host, their span of life being from 3 to 8 years. The females lay a vast number of eggs, a Necator about 10,000 a day and an Ancylostoma about 20,000.

The rhabditiform larva which hatches out of the egg has a well-developed alimentary tract with a long buccal cavity, a bulbous esophagus, a gut, and an anal opening.

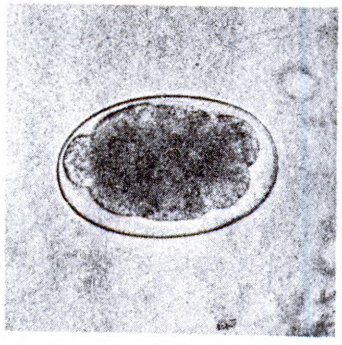
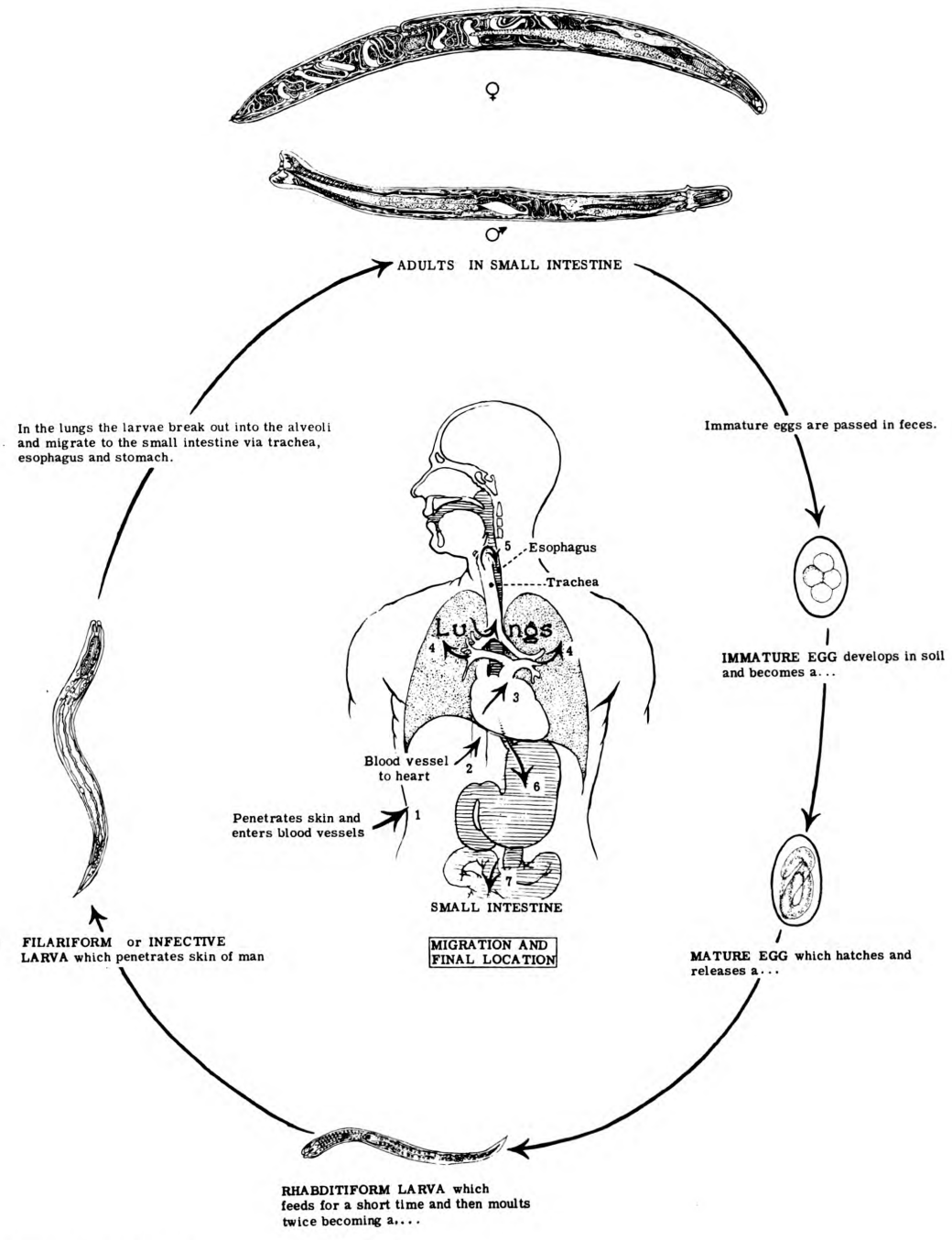


Fig. 65 - Egg of hookworm

LIFE CYCLE OF HOOKWORMS



NAVAL MEDICAL SCHOOL

Fig. 66

It feeds voraciously on organic debris for about 3 days, grows up to 0.5 mm., and molts. On the fifth to eighth day the larva stops feeding, the mouth closes, the esophagus elongates, and, after a second molting, it becomes filariform and capable of penetrating the skin and infecting a new host. Reaching the intestine via the heart and lungs, the larvae develop to adulthood and begin to lay eggs in about 5 weeks.

Ancylostoma braziliense, Toxocara canis and T. cati

The adults of these species, normally parasitic in dogs and cats, are not important intestinal parasites of man. The larvae of the hookworm, A. braziliense, however, invade the skin to cause a severe creeping eruption or cutaneous larva migrans by their extensive tunneling in the deep epithelial layers. The larvae of the ascarid genus, Toxocara, invade the extra-intestinal viscera, producing a pathological condition called visceral larva migrans.

Geographic Distribution: Tropical and subtropical regions of the world.

Morphology

A. braziliense is the smallest species of the genus, males measuring 7.5 to 8.5 mm. and females, 9 to 10.5 mm. in length. The buccal capsule has one pair of small median teeth and one of large outer teeth. Eggs and larvae are similar to those of A. duodenale.

The ascarids are much larger, adult males of both species being 4 to 6 cm. long and females varying from 4 to 12 cm. The second-stage larvae in host-cell capsules of the viscera measure about 0.4 mm. in length by 0.016 to 0.019 mm. in width.

Life Cycle

A. braziliense follows the developmental pattern of the human hookworms. Separate strains appear to exist in cats, dogs and man. The human strain is limited in distribution and always produces intestinal hookworm infection in man. The canine and feline strains, which are cosmopolitan, are apparently unable to penetrate the subdermal layers of the human skin. When man is exposed to moist, shady soil where cats or dogs infected with A. braziliense have previously defecated, the filariform larvae penetrate the skin. A serpiginous tunnel is produced in the dermal layers at the rate of several millimeters to a few centimeters a day. The lesion is at first inflamed, then elevated and vesicular, and finally dry and crusted. The larvae may continue their activity for weeks but very rarely reach the circulation.

Toxocara infections are contracted in typical ascarid style, when man ingests the infective-stage eggs. Visceral larva migrans results when the hatched larvae invading the extra-intestinal viscera are blocked from further development by the formation of host-cell capsules in which they may remain alive for some time.

Ascaris lumbricoides

Geographic Distribution: A. lumbricoides has a cosmopolitan distribution.

Morphology

Adults - These are large worms with the females measuring from 20 to 35 cm., or even up to 45 cm., in length and about 5 mm. in diameter; the males are roughly two-thirds as long and more slender. Both sexes are provided with three finely denticulate lips, each having a pair of minute papillae. The male has a curled tail and two copulatory spicules but no gubernaculum. The female has paired tubular genital organs coiled through the middle and posterior thirds of the body. The vulva is near the junction of the anterior and middle thirds.

Eggs - The eggs are very characteristic, having a thick, transparent inner shell covered by an irregular, wrinkled albuminous coat which is stained golden brown by the bile pigment. They are ovoid in shape with a length of 45 to 75 microns and a width of 35 to 50 microns. Unfertilized eggs are not uncommon; they are more elongate, about 90 by 40 microns, and have an amorphous mass of refractile granules instead of the well-defined spherical zygote of the fertilized eggs. Both fertile and infertile eggs without the outer wrinkled coat occasionally may be found. These decorticated eggs closely resemble hookworm eggs, but they may be distinguished by the thicker shell, very thin in hookworm ova, and the undivided cell mass which usually is in 4- or 8-cell stage in the hookworms.

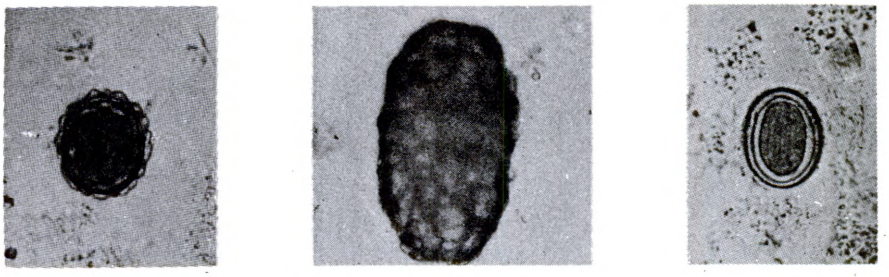
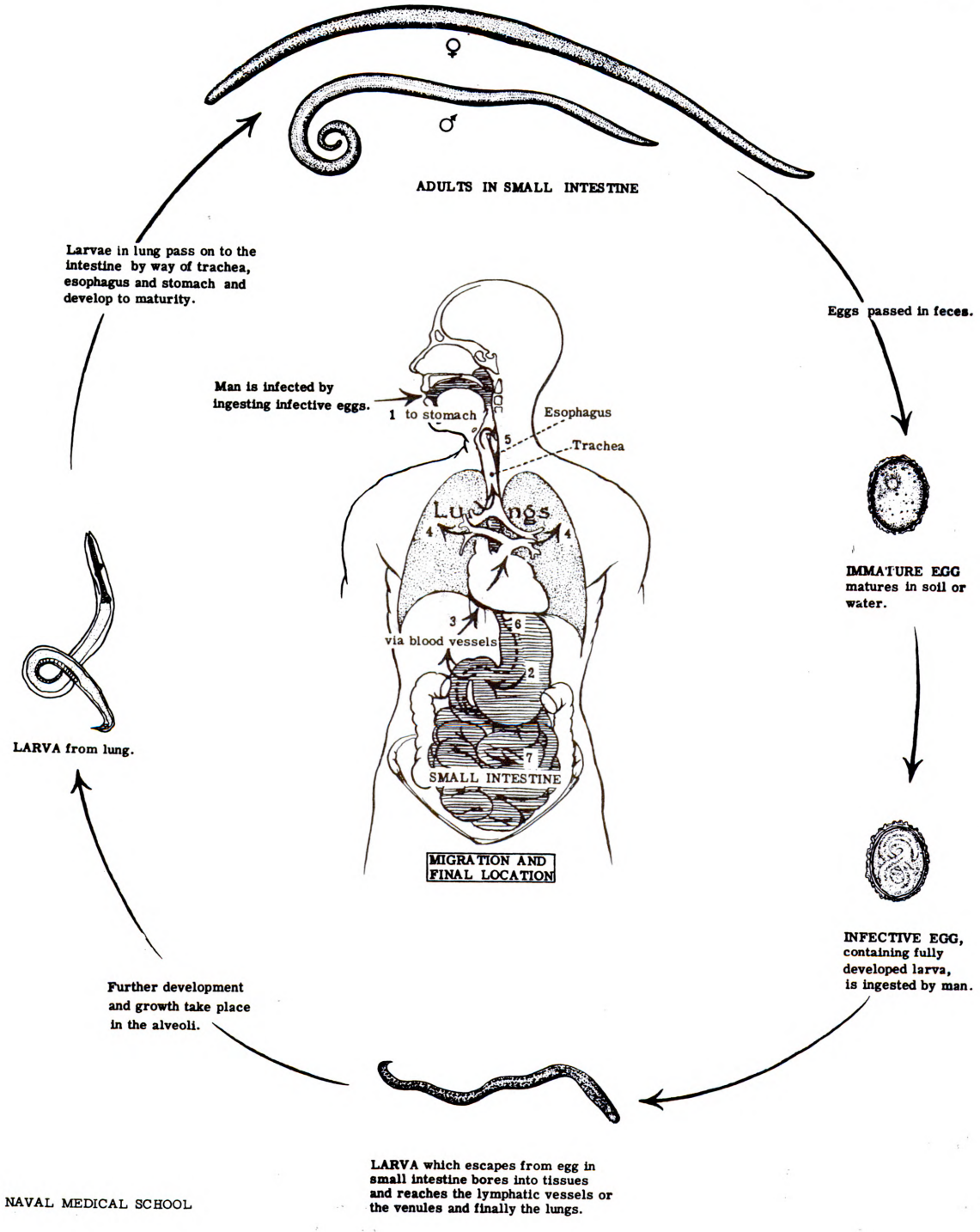


Fig. 67 - Eggs of Ascaris lumbricoides

Life Cycle (Fig. 68)

No intermediate host is needed. The eggs when passed are unsegmented and must develop to larvae before they are infective. Under favorable conditions with a temperature of 23° to 33° C. and adequate moisture and oxygen, an active embryo may develop in 10 days, but the majority of eggs require about 3 weeks under natural conditions. When deposited in unfavorable sites, the eggs may remain dormant but viable for long periods, 2 to 4 years, and then develop when better conditions arise. They are very resistant to desiccation and low temperatures. The shell is extraordinarily impermeable; the embryos will develop in 5% formalin or 1% sodium hydroxide. Immature eggs are not affected by the digestive juices and will pass through the gastro-intestinal tract unharmed.

LIFE CYCLE OF ASCARIS LUMBRICOIDES



NAVAL MEDICAL SCHOOL

Fig. 68

After ingestion of embryonated eggs, the digestive juices act on the egg shell and liberate the larva in the small intestine. The larvae migrate through the intestinal mucosa and are carried via the lymphatics and blood vessels to the liver, then to the heart, and finally to the lungs. Here they leave the circulatory system to rest in the air spaces for about 10 days during which time they undergo two molts and reach a length of about 2 mm. The migration through the lungs causes considerable damage in the alveoli and bronchioles and, in cases of heavy infections, may give rise to serious pulmonary symptoms.

Trichuris trichiura

The generic name for this worm is in dispute and has not been ruled on by the International Commission on Zoological Nomenclature. Trichuris is used in this discussion because that is the preference of the American Society of Parasitologists. Trichocephalus trichuris is the name used by some authors. Because of the shape of this worm, it is commonly known as the whipworm.

Geographic Distribution

Cosmopolitan; more common in warm, moist regions of the world.

Morphology

The worms are attenuated in their anterior three-fifths and fleshy in the posterior portion. The esophagus is a delicate capillary tubule capable of dilation at its distal end. A set of muscles which operate a stylet are present at the anterior end. The male measures 30 to 45 mm. in length and has its caudal extremity coiled through as much as 360° or more. A single lanceolate spicule protrudes through a retractile penial sheath which has a bulbous termination covered with many small recurved spines. The female measures 35 to 50 mm. in length, is bluntly rounded at the posterior end, and has a single ovary. The vulva is at the anterior extremity of the fleshy portion. The eggs measure 50 to 54 microns by 22 to 23 microns. They are barrel-shaped and have a double shell, the outer one usually bile-stained. They also have unstained bipolar prominences which have the appearance of mucoid plugs.

Fig. 69 - Egg of Trichuris trichiura

Life Cycle (Fig. 70)

The adult worms live attached to the wall of the human cecum, less commonly the appendix or colon. Man is the only proven host although the whipworms obtained from the pig and from certain monkeys are morphologically similar. The eggs are discharged from the female worm and passed with the feces in the unsegmented stage. They require at least 10 to 14 days in the soil for embryonation.

LIFE CYCLE OF TRICHURIS TRICHIURA

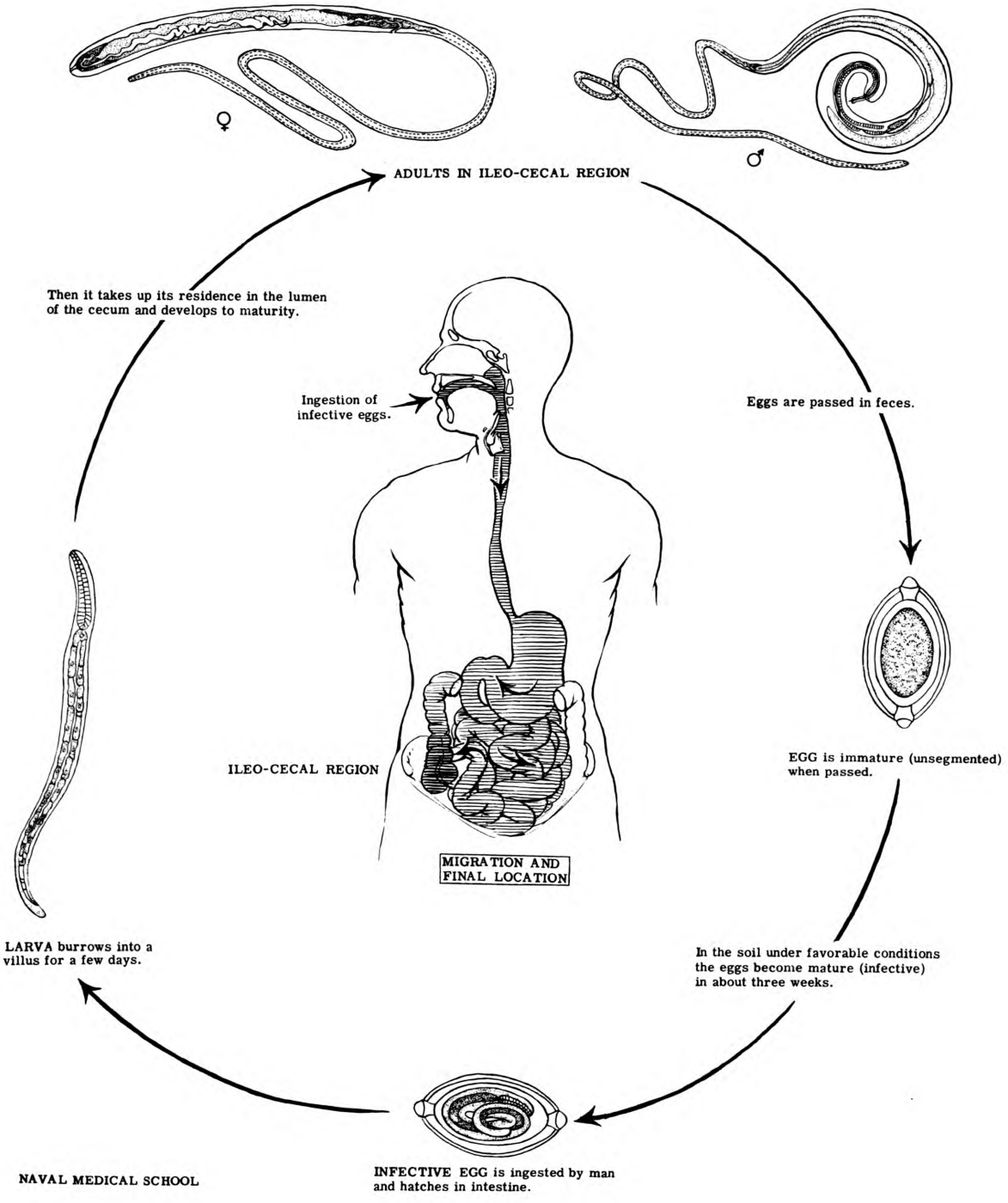


Fig. 70

Moisture is essential for their development. Man becomes infected upon swallowing the fully embryonated eggs. The shell is digested in man's small intestine, and the emerging larva becomes temporarily attached to the wall of the small bowel. Here it obtains nourishment, later passing to the site of adult attachment. About 3 months are required for a worm to become mature.

Enterobius vermicularis

This worm was formerly placed in the genus Oxyuris. The common names for the worm are pinworm, seatworm, and threadworm.

Geographic Distribution

This worm is world-wide in its distribution. In the United States it is the most common human helminth.

Morphology

The adult worms are more or less spindle-shaped. The oral end lacks a true mouth capsule but is provided with three lips and a pair of laterally placed cephalic alae or "wings." The male is from 2 to 5 mm. in length and 0.1 to 0.2 mm. in greatest diameter. Its posterior end is strongly curved ventrad. It has a single conspicuous copulatory spicule which is about 70 microns long. The bursa is greatly reduced but has well-developed caudal "alae." The female is from 8 to 13 mm. in length and 0.3 to 0.5 mm. in greatest diameter. The tail portion, beyond the anus, is distinctly attenuate; it constitutes about one-third of the entire length. The vulva opens ventrally just beyond the first third of the body. The vagina is long and extends posteriorly some distance from the vulva before it joins the paired genital organs consisting successively of uteri, oviducts, and ovarian tubules which are coiled back and forth several times in the middle of the body. The uteri are tremendously distended in the gravid female so that the entire body is packed with eggs. The eggs are elongate, ovoid, flattened on one side, and measure 50 to 60 by 20 to 30 microns.

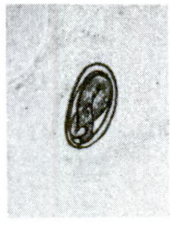
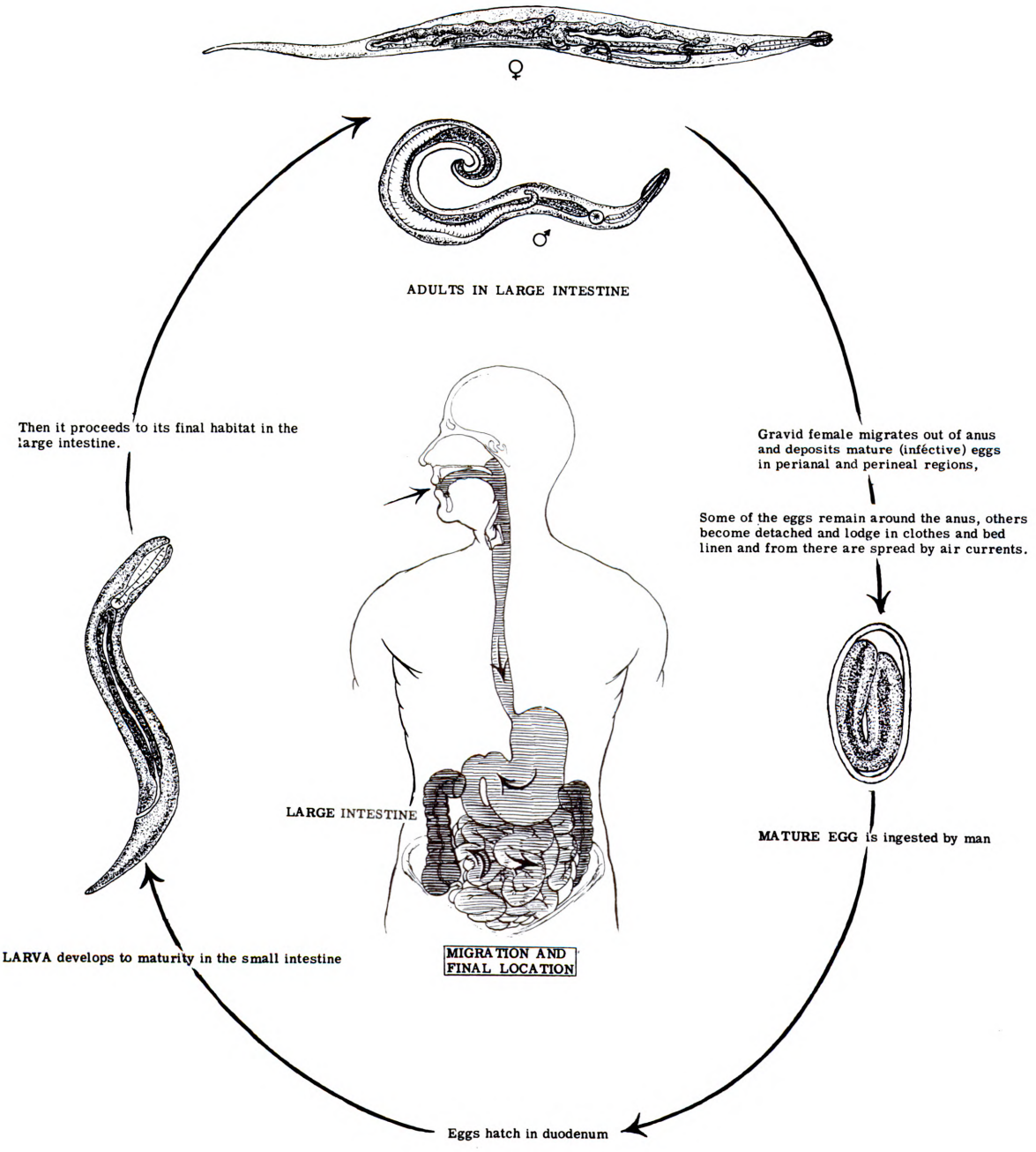


Fig. 71 - Egg of Enterobius vermicularis

Life Cycle (Fig. 72)

The adult worms inhabit the cecum and adjacent portion of the intestine. The pressure of the eggs on the esophagus of the gravid female causes the worm to release her attachment; she migrates from place to place in the lumen of the intestine, commonly passing down the colon and out the anus to the perianal and perineal skin, frequently entering the vagina in females. In these areas the female oviposits and dies. The eggs require no intermediate host for their subsequent development and are usually infective shortly after being deposited by the worm. The activity

LIFE CYCLE OF ENTEROBIUS VERMICULARIS



NAVAL MEDICAL SCHOOL

Fig. 72

of the females outside the anus usually creates an intense itching which provokes scratching of the area. Thus eggs get on the fingers or under the fingernails and are carried to the mouth to be swallowed. Infection may result from contact with contaminated bed linen. Since the eggs are light and float in air, infection is possible through inhalation.

LABORATORY DIAGNOSIS OF INTESTINAL NEMATODE INFECTIONS

The laboratory diagnosis of intestinal nematodes is most practically made by finding and identifying the characteristic ova or larvae, rarely adults, in the feces. Two rather rare parasites of man, Trichostrongylus and Physaloptera, have been included in this discussion for the sake of completeness. Occasionally the urine or sputum may be examined. The ova of Ancylostoma, Necator, Ascaris, Trichuris, Ternidens, Physaloptera, and Trichostrongylus will be found in the feces; rarely Enterobius eggs will also be found, and ova of Strongyloides may appear after brisk purgation or in severe diarrhea. The feces should be examined by direct smear, flotation, and centrifugation. Sedimentation is rarely used for the nematode ova. Flotation methods offer the highest return by the simplest procedure, adequate for the majority of cases infected with these helminths. Light infections which tend to be overemphasized as a cause of illness, may be missed. These light infections may be discovered by using a combination of flotation and centrifugation. These combined methods, however, require much more apparatus and time. Specimens may be preserved and examined in the laboratory at a later date.

In using flotation methods, it must be remembered that the ova are of different weights, and the specific gravity of the solution should be adequate to lift them. The infertile Ascaris egg is the heaviest, requiring a solution of specific gravity of about 1.250; some infertile ova may be raised by specific gravities of 1.200. The critical specific gravity for hookworm is about 1.055, for whipworm about 1.150, and for fertile Ascaris about 1.130. Best yields are obtained with a specific gravity of at least 1.180 or higher.

The bile-stained ova of Trichuris and normal Ascaris are easily identified, but the transparent-shelled eggs of the hookworms, the decorticated ova of Ascaris, and the more rarely found ova of Trichostrongylus, Enterobius, Ternidens, and Strongyloides require careful differentiation. The criteria most commonly used are the size, shape, thickness of shell, and stage of development of the embryo. The two hookworms are practically indistinguishable by their ova, those of Ancylostoma usually being considered shorter; culture may be used and identification made from the larval stages.

Ova of two accidental parasites, Heterodera marioni and Capillaria hepatica, have been found in the feces of man several times. The eggs of H. marioni, a parasite of the roots and stems of edible plants, may be mistaken for those of hookworms or Enterobius. Capillaria hepatica, a parasite in the liver of rats and other rodents, has ova which are similar to those of Trichuris.

EGGS OF INTESTINAL NEMATODES

SPECIES	SIZE (in microns)	SPECIAL FEATURES
Strongyloides stercoralis	50-58 x 30-34; average 55 x 32	Oval, colorless, thin shell; developed to larval stage
Ancylostoma duodenale	56-60 x 35-40; average 60 x 38	Oval, colorless, thin shell; poles bluntly rounded; usually developed to 4-cell stage
Necator americanus	64-76 x 35-40; average 70 x 38	
Trichuris trichiura	50-54 x 22-23; average 52 x 23	Barrel-shaped, brown, thick shell; mucoid plug at each pole; unsegmented
Ascaris lumbricoides	45-75 x 35-50; average 60 x 45	Oval; outer wall mammillated, brown; inner wall thick, clear; unsegmented
Infertile	88-95 x 40-45	Irregular, rhomboidal; granular contents
Decorticated	43-68 x 33-48	With only clear, thick inner wall
Trichostrongylus species	75-95 x 40-55; average 89 x 48	Oval, colorless, thin shell; one pole more pointed; usually morula stage
Ternidens deminutus	Average 80 x 50	Oval, colorless, thin shell; poles broadly rounded; developed beyond 4-cell stage
Enterobius vermicularis	50-60 x 20-30; average 55 x 26	Oval, colorless, thin shell; flattened on one side; developed to larval stage
Physaloptera caucasica	Average 50 x 35	Oval, colorless, thick smooth shell; developed to larval stage
Capillaria hepatica	50-65 x 30-35	Barrel-shaped; brown, distinctly radially pitted, thick shell; unsegmented
Heterodera marioni	80-120 x 25-45	Bean-shaped, colorless, thin shell; air space at poles; developed to morula stage

Larvae in feces are almost certainly Strongyloides; in old or constipated stools the larval stages of the hookworms or Trichostrongylus may have hatched from the eggs, and in rare instances free-living coprophagous forms (Rhabditis) may contaminate the specimen. Methods used should include direct smears and centrifugation. Good results have also been obtained with the zinc sulfate flotation-centrifugation method, but flotation technics alone are usually unsatisfactory. Culture methods and the Baermann apparatus are also of definite value in diagnosing suspected infections where larvae may be found.

RHABDITIFORM LARVAE OF INTESTINAL NEMATODES

Characters	Strongyloides stercoralis	Ancylostoma and Necator
Size (in microns)	200-250 (in stool); usually 225	250-300 (when hatched); usually 275
Buccal Cavity (length)	10 microns (shorter than body width)	20 microns (longer than body width)
Genital Primordium	Large; about 35 microns	Small; about 20 microns
Tail Tip	Short; bluntly pointed	Short; sharply pointed

Trichostrongylus and Rhabditis larvae resemble the hookworms in having buccal cavities which are considerably longer than the body width and small genital rudiments. The esophagus of Rhabditis is pseudorhabditiform, having a median swelling in addition to the terminal bulb. Trichostrongylus is distinguished by its long filamentous tail.

When culture methods are used, or very rarely in the stools, filariform as well as the earlier larval stages may be found. The esophagus of the infective larva of the hookworms is not typically filariform; a more correct term is pseudofilariform. The pseudofilariform larva of Trichostrongylus has a tuberculated tail; there is no filariform larva in Rhabditis.

Although seldom necessary, Ancylostoma duodenale, Necator americanus, and the other hookworms can be differentiated by their filariform larvae. Those of A. duodenale have a slightly flattened head and a rather narrow esophagus without a definite constriction at the esophageal-intestinal junction; also the esophageal spears are inconspicuous and unequal. In N. americanus the head is smaller and more cylindrical, and the esophagus is broader with a marked constriction where it joins the intestine; the esophageal spears are conspicuous and equal. The tail of A. braziliense is more slender than in the other two species.

For the diagnosis of Enterobius infection, the collection of ova or adult females from the perianal folds is the method of choice. The NIH swab is a time-tested

technic which gives excellent results. The scotch tape method is a more recently devised technic that has many advantages. Ova may also be collected from other regions of the body, from clothing, and from furniture in homes containing infected persons by using the transparent adhesive tape; ova may also be recovered from fingernail scrapings. In about one-half of the cases of ascariasis and one-sixth of trichuriasis, they may be collected from the perianal region.

FILARIFORM LARVAE OF INTESTINAL NEMATODES

Characters	Strongyloides stercoralis	Ancylostoma and Necator
Size (in microns)	500-700	About 800
Genital Primordium	Behind middle of body	Near middle of body
Esophagus - Length	About two-fifths body length; narrow throughout length	About one-fourth body length; slight posterior bulb
Tail Tip	Minutely notched	Pointed

Adult intestinal nematodes are sometimes evacuated spontaneously or after treatment and may then be identified by their anatomical features (see key, page 186). The smaller worms may require special methods of recovery. Quite often Enterobius females may be scraped from the perineum. Ascaris adults tend to migrate and have been taken from the nostrils and mouth in numerous cases.

Larvae of species which migrate through the lungs and respiratory passages (Strongyloides, the hookworms, Ascaris) may very rarely be recovered from the sputum. The direct smear and centrifugation may be employed in demonstrating them. In a few rare instances larvae or eggs have been found in the urine. Some workers have reported greater success in finding Strongyloides larvae by using a duodenal tube, suggesting that they are destroyed or digested as they pass out with the feces; other statistics show only a slight increase in positives by this method. Duodenal drainage is not recommended except in cases where the feces are negative, and there is strong suspicion of Strongyloides infection.

It may be desirable to recover intestinal roundworm larvae or ova from the soil. The Baermann apparatus is an ingenious and practical device for obtaining the larvae of hookworms, Strongyloides, and, it should be emphasized, free-living nematodes. Other methods may be used to recover eggs from the soil.

Serological methods for the diagnosis of intestinal nematode infections exist but are of little practical significance.

TRICHINOSIS

Trichinella spiralis

Geographic Distribution

This parasite has a world-wide distribution. The principal reservoir for human infection is the pig; the parasite is, therefore, most common among pork-eating people whose food habits allow the consumption of raw or insufficiently cooked pork. It is practically unknown in people of the Mohammedan and Jewish faiths. The incidence of infection in the United States, as determined by postmortem examination of diaphragms, is about 17%. The importance of this parasite in the United States is frequently underestimated.

Morphology

The adults are small white worms, just visible to the unaided eye, the male being 1.5 mm. long and the female about 3.9 mm. The stout posterior half of the male is filled mostly with testes. The posteriorly located cloaca, eversible during copulation, is guarded by two cone-shaped papillae which clasp the female during this act. The rounded posterior half of the female contains the ovary and becomes distended as the eggs develop. The vulva is anterior in position, a location which facilitates the extrusion of the larvae into the tissue of the intestine by the burrowing female. The larvae, when extruded by the female, are about 100 microns long and 6 microns in diameter, a size which permits transit through the capillaries. Upon arrival in a suitable muscle fiber, the larva coils up, grows rapidly, and becomes differentiated sexually.

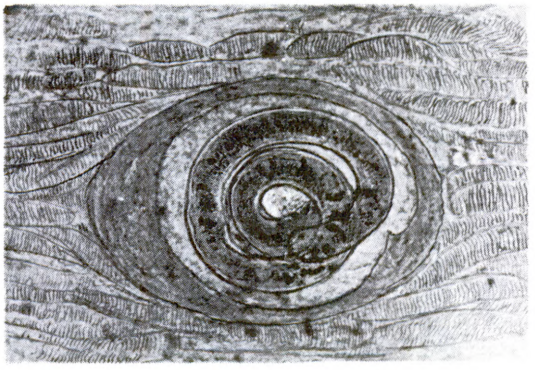


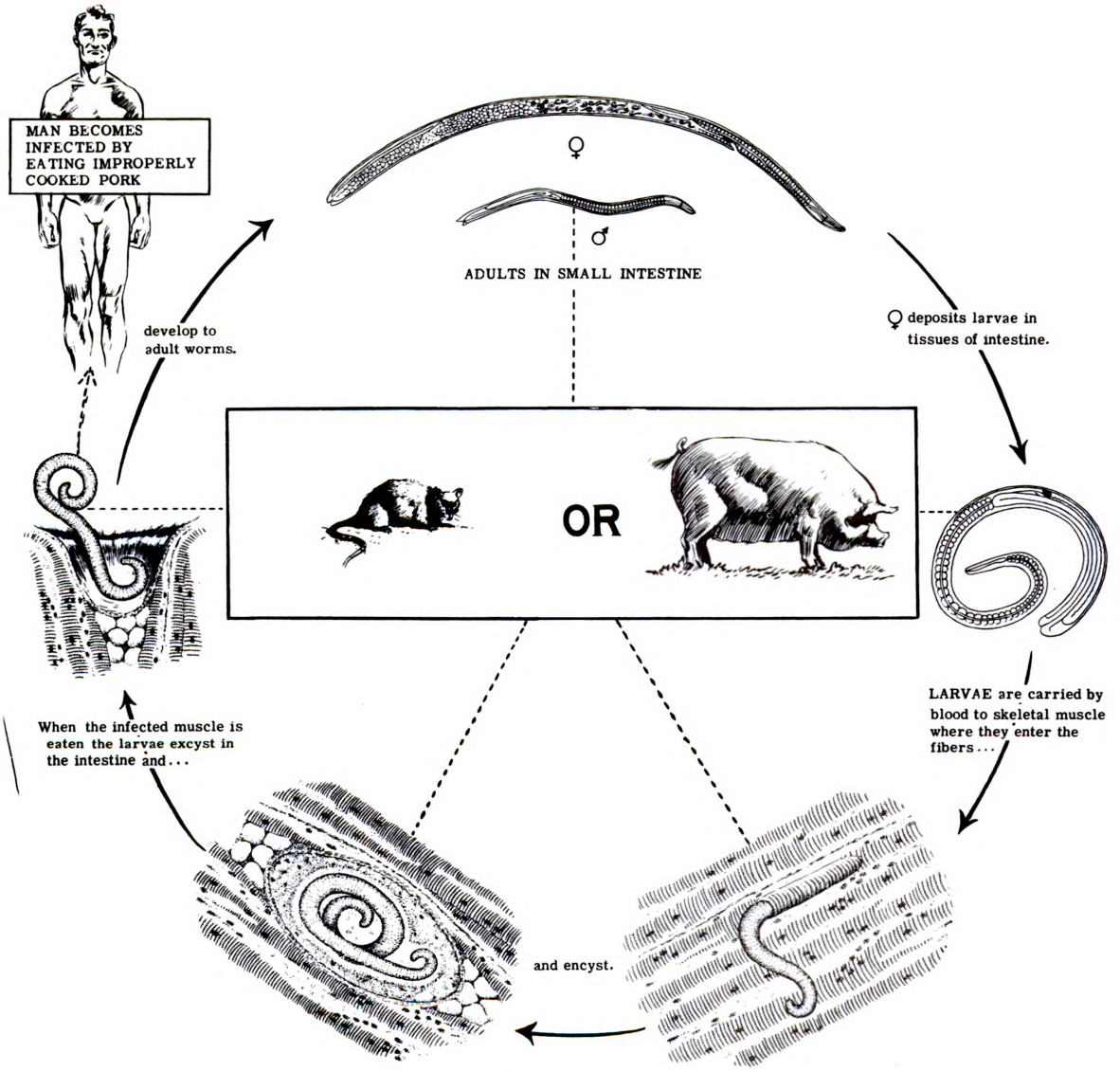
Fig. 73 - Larva of Trichinella spiralis encysted in striated muscle

Life Cycle (Fig. 74)

While both adults and larvae develop within the same host, two hosts are required to complete the life cycle. In nature the infection is normally propagated by the black and brown rats which are cannibalistic. Pigs, wild boars, bears and other mammals which eat flesh become secondarily infected. Recently Trichinella has been found in walrus meat in Greenland. The relation between rats, swine, and man is indicated (Fig. 74).

The female is viviparous, depositing larvae and not eggs, a single female giving birth to at least 1,500 larvae over a period of 6 weeks. Most of these larvae reach the portal or lymphatic circulation and ultimately the heart, being distributed from there by the systemic circulation. They may become lodged in various tissues of the body, but they are capable of further development and encapsulation only in voluntary (skeletal) muscle. The greatest invasion occurs in those muscles with the richest blood supply, such as the diaphragm and intercostal, laryngeal, tongue, and eye muscles. The larva is provided with a spear, a movable, boring apparatus at the anterior end, by which it is aided in its penetration of muscle fiber. It comes

LIFE CYCLE OF TRICHINELLA SPIRALIS



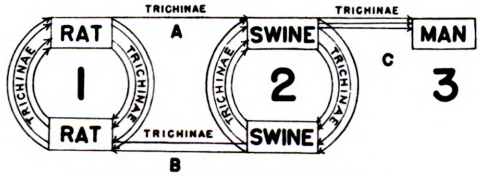


DIAGRAM SHOWING TRANSFER OF TRICHINAE FROM HOST TO HOST

NAVAL MEDICAL SCHOOL

Fig. 74

to lie along the long axis of the fiber, grows to about 1 mm. in length in 10 to 14 days, and assumes the spiral form of the encysted parasite. The invaded muscle fiber rapidly undergoes degeneration. The capsule is an ellipsoidal sheath with blunt ends resulting from the infiltration of protective cells of the host. This is an attempt on the part of the host to wall off the invading larva.

LABORATORY DIAGNOSIS OF TRICHINOSIS

An early laboratory diagnosis is extremely difficult and rarely made. During the first few days after infection, adult worms may be found occasionally by sedimentation of the feces. After the sixth day and until the end of the migration period, larvae may be demonstrated in the blood. This is of value only in heavy infections, yielding about two larvae per milliliter of blood. Larvae may also be recovered from the spinal fluid. Biopsy for encysted forms is most practically done upon the deltoid, biceps, or gastrocnemius muscles. Compression and digestion methods are used; care must be exercised so as not to be misled by old infections.

Serological and allied tests are not sufficiently specific to be more than an adjunct to the clinical diagnosis. The skin test becomes positive earliest but is of no value before the twenty-first day; very light and very heavy infections may not be detected by this method. False positives occasionally occur as do nonspecific reactions due to diluting fluid. After the fourth week, precipitation and complement fixation tests become useful. The specificity of precipitation is about the same as that of the skin test. The increased accuracy of complement fixation seldom justifies the complexity of the procedure, and the test is not often used.

Postmortem examination includes digestion, compression, and sectioning methods. Small bits of diaphragm give the highest percentage of positives, but other striated muscles should also be examined.

TISSUE ROUNDWORMS

Life Histories

This group includes the worms of which the adult stages are found in tissues rather than in the intestinal tract. It includes the filarial worms (filariae) and the guinea worm. The life histories of the filariae differ fundamentally from that of the guinea worm and will be considered separately.

Adult filarial worms live in the tissues or body cavities of vertebrate hosts where, instead of laying eggs, they give birth to young which are called microfilariae (Fig. 80). The microfilariae, which may be in the peripheral blood vessels or the subcutaneous lymph spaces, are picked up by the intermediate host with its blood meal. This host, in all cases, is a dipterous insect, there being

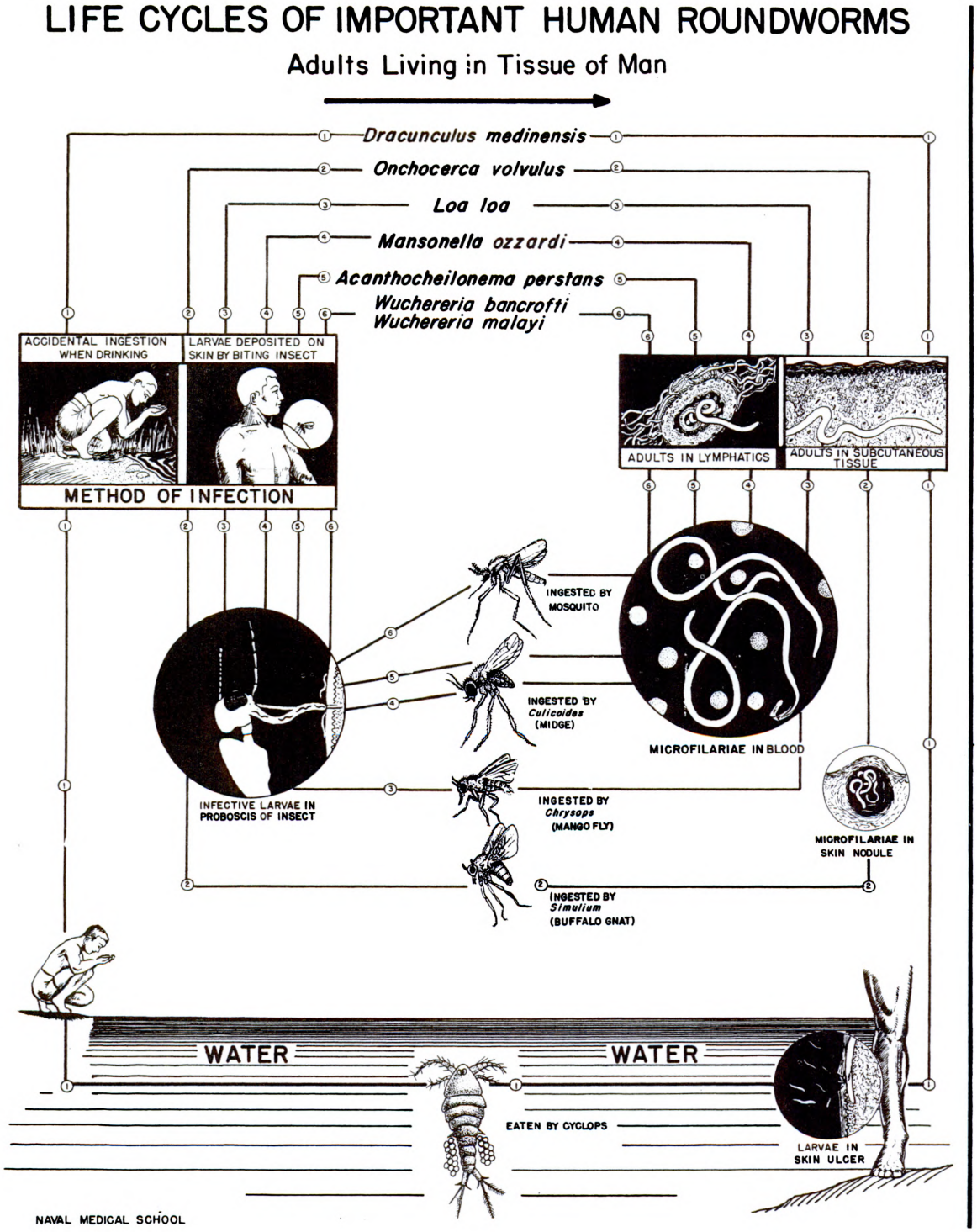

LIFE CYCLES OF IMPORTANT HUMAN ROUNDWORMS
Adults Living in Tissue of Man
Dracunculus medinensis
Onchocerca volvulus
Loa loa
Mansonella ozzardi
Acanthocheilonema perstans
Wuchereria bancrofti
Wuchereria malayi
ACCIDENTAL INGESTION WHEN DRINKING
LARVAE DEPOSITED ON SKIN BY BITING INSECT
METHOD OF INFECTION
ADULTS IN LYMPHATICS
ADULTS IN SUBCUTANEOUS TISSUE
INGESTED BY MOSQUITO
INGESTED BY Culicoides (MIDGE)
MICROFILARIAE IN BLOOD
INFECTIVE LARVAE IN PROBOSCIS OF INSECT
INGESTED BY Chrysops (MANGO FLY)
MICROFILARIAE IN SKIN NODULE
INGESTED BY Simulium (BUFFALO GNAT)
WATER
WATER
EATEN BY CYCLOPS
LARVAE IN SKIN ULCER
NAVAL MEDICAL SCHOOL

Fig. 75

a specific insect or insects for each species of filarial worm. A fairly uniform development within the insect occurs in all filariae. The microfilaria sheds its sheath, penetrates the gut wall of the insect, and migrates to the thoracic muscles where it passes through at least two molts before becoming infective. It then migrates to the proboscis of the insect. The infective larva leaves the proboscis when the insect bites, enters man, and develops to maturity in the tissues of its preference.

The adult female guinea worm lives in subcutaneous tissues of the body. She gives birth to living larvae which are deposited in small surface ulcers. These ulcers burst upon contact with water and free the larvae which are ingested by a crustacean, Cyclops. They bore through the stomach wall of the crustacean and remain in the hemocoele where they molt and become infective. Man is infected by drinking water containing the infected Cyclops. The infective larva escapes from the crustacean in the stomach of man, penetrates the intestinal wall, and wanders in the body tissue.

Wuchereria bancrofti

Geographic Distribution (Fig. 76)

Wuchereria bancrofti, the causative agent of filariasis and filarial elephantiasis, has a wide distribution in tropical and subtropical countries.

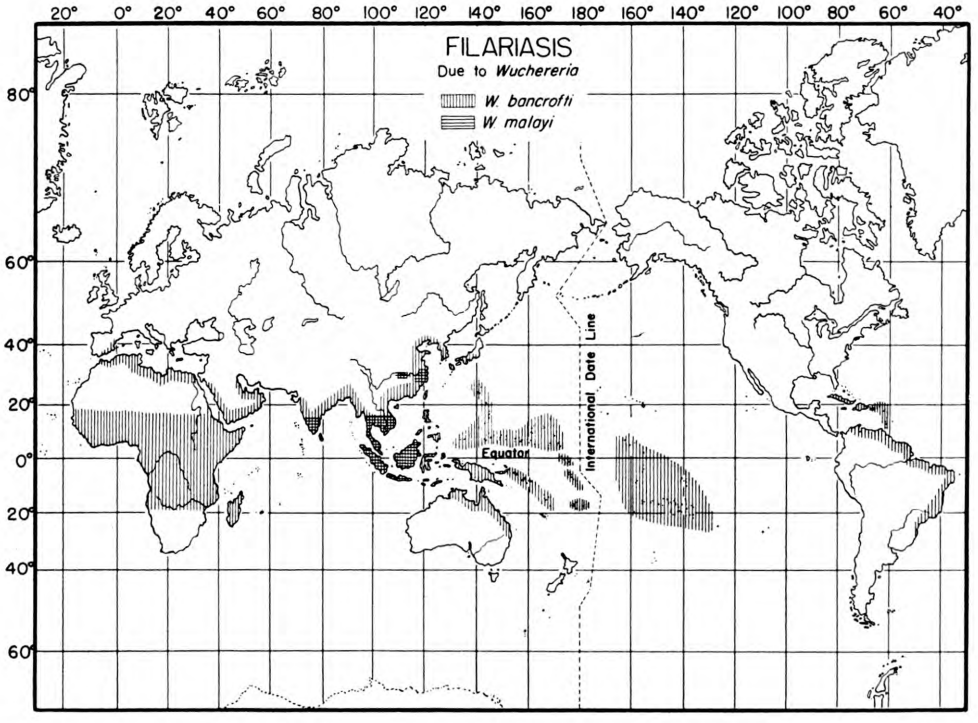


Fig. 76 - Geographical distribution of Wuchereria bancrofti and W. malayi

Morphology

The adults are white thread-like worms with a smooth cuticula. The head is provided with two rows of small papillae. The small unarmed oral opening leads directly into the esophagus, there being no mouth cavity. The male is about 4 cm. long and has a sharply curved tail and two unequal spicules. The female is about twice as long as the male. The reproductive organs are paired. The vulva opens anteriorly, about 1 mm. from the mouth.

The microfilariae are about 300 microns long and 7 microns in diameter. The anterior end is rounded and the posterior tapering. They are encased in a sheath which is so close-fitting that it is detectable only as it projects beyond the head or tail.

Fig. 77 - Microfilaria of Wuchereria bancrofti

Life Cycle (Fig. 78)

1. Man is the only known definitive host.

2. While complete larval development to the infective stage has been demonstrated in many species of mosquitoes, many of them are of little or no importance as vectors. The most important vectors are Culex quinquefasciatus, a cosmopolitan night-biting mosquito, and Aedes pseudoscutellaris, a day-biting mosquito in the islands of the Pacific. Many anopheline species are also vectors.

3. In order to infect man, the microfilaria must undergo a metamorphosis in the mosquito to the infective stage which requires from 6 to 20 days.

4. The microfilariae are present periodically in the peripheral blood of man. This periodicity, which may be nocturnal, diurnal, or a combination of the two, is closely associated with the biting habits of the mosquito vector. There is some evidence to indicate that the nonperiodic type is a modification of those showing nocturnal periodicity.

Wuchereria malayi

Geographic Distribution (Fig. 76)

Morphology

The adults are similar to those of W. bancrofti. The ensheathed microfilariae are 177 to 260 microns long, 5 to 6 microns in diameter, and have nuclei which extend to the tip of the tail.

LIFE CYCLE OF WUCHERERIA BANCROFTI

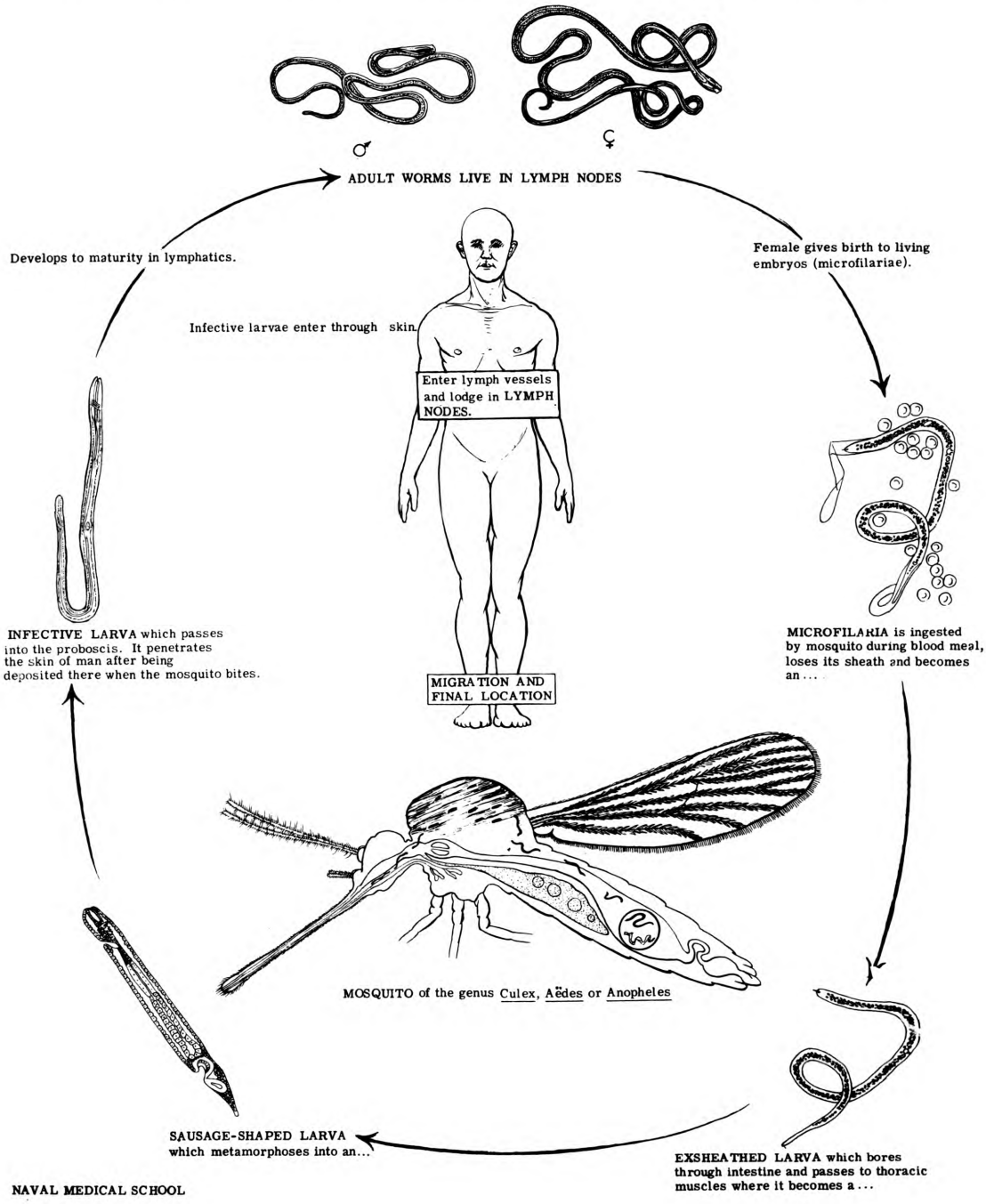


Fig. 78

Life Cycle

This is believed to be almost identical with that of W. bancrofti. The intermediate hosts are mosquitoes of the genera Mansonia, Anopheles, and Armigeres.

Loa loa

Geographic Distribution

Tropical West Africa, particularly in the Congo River country.

Morphology

Adults - The adults are white thread-like worms. The cuticula is covered with chitinous wart-like nodules. The head has two lateral and four small median papillae. The male is about 3 cm. long; the tail end is tapered and curved and has two spicules of unequal length. The female is about 6 cm. long.

Microfilariae - The microfilariae of L. loa closely resemble those of W. bancrofti. Two differential points are usually quite obvious in the stained preparations:

1. The angular, ungraceful curves of the microfilariae of L. loa as compared with the smooth, graceful curves of W. bancrofti.

2. Nuclei extending to the tip of the tail in L. loa, while the tail of W. bancrofti has no terminal nuclei.

Life Cycle (Fig. 75)

Man is the only definitive host. This filaria lives in the subcutaneous tissue, but the microfilariae find their way into the blood stream. Here they appear with diurnal periodicity. The intermediate host is a fly (deer fly, mangrove fly) of the genus Chrysops. Development within the fly and the escape of the infective larval filariae to new hosts closely follow that of W. bancrofti in the mosquito. The development of the filaria in the human host is slow, several years being required for it to attain maturity.

Onchocerca volvulus

Geographic Distribution

This worm is found in the tropical belt of West Africa, particularly in the Congo area, and on the Pacific slope of Guatemala and southern Mexico.

Morphology

Adults - The adults are white filiform worms with distinct annular striations of the cuticula. At the anterior end there are eight small papillae in two circles and a

pair of large oval papillae. The male is 2 to 4 cm. long and has a tightly curved tail with two spicules of unequal length. The female is about 10 times as large as the male. The vulva is anterior, less than 1 mm. from the head end.

Microfilariae - Two sizes of microfilariae occur, one about 250 microns, the other about 325 microns, probably representing male and female. Both the anterior end and the tail are free of nuclei.

Life Cycle (Fig. 75)

The adults, and the microfilariae as well, normally have their habitat in the connective tissue under the skin. Visible fibrous tumors usually form around the adults. The female is embedded in the connective tissue tumor except for the anterior end with its vulva which, together with the accompanying males, lies free in the liquid center, an arrangement which permits fertilization. The microfilariae, leaving their sheaths when born, migrate from the parental prison to the surrounding subcutaneous connective tissue. They may wander from nodules on the head into the cornea of the eye, causing blindness. In the insect host, Simulium (the black gnat, the jinja fly of Africa), the migration and development of the microfilariae to the infective forms and the escape to a new host parallel that of W. bancrofti in the mosquito. Man is the only known definitive host, although closely related species of Onchocerca occur in other animals.

Acanthocheilonema perstans

This filaria is quite common in the tropical regions of Africa and South America. It apparently is a harmless parasite although some workers associate it with edema and abscess formation.

Mansonella ozzardi

This filarial worm occurs only in the Western Hemisphere, having been found in Central America, South America, and in certain of the Caribbean Islands. Apparently it is a harmless parasite.

Dracunculus medinensis

The guinea worm, the dragon worm

Geographic Distribution

The important endemic areas are in the Nile Valley (excluding Egypt), west coast of Africa, parts of Arabia, and India. There are indications that infection by Dracunculus in the Middle East is on the decline. Infected persons have carried it over to some of the Caribbean Islands, the Guianas, and northern Brazil. It is apparently present in fur-bearing animals in the United States.

Morphology

Adults - The adult females are cord-like worms with a smooth cuticula. The

LIFE CYCLE OF DRACUNCULUS MEDINENSIS

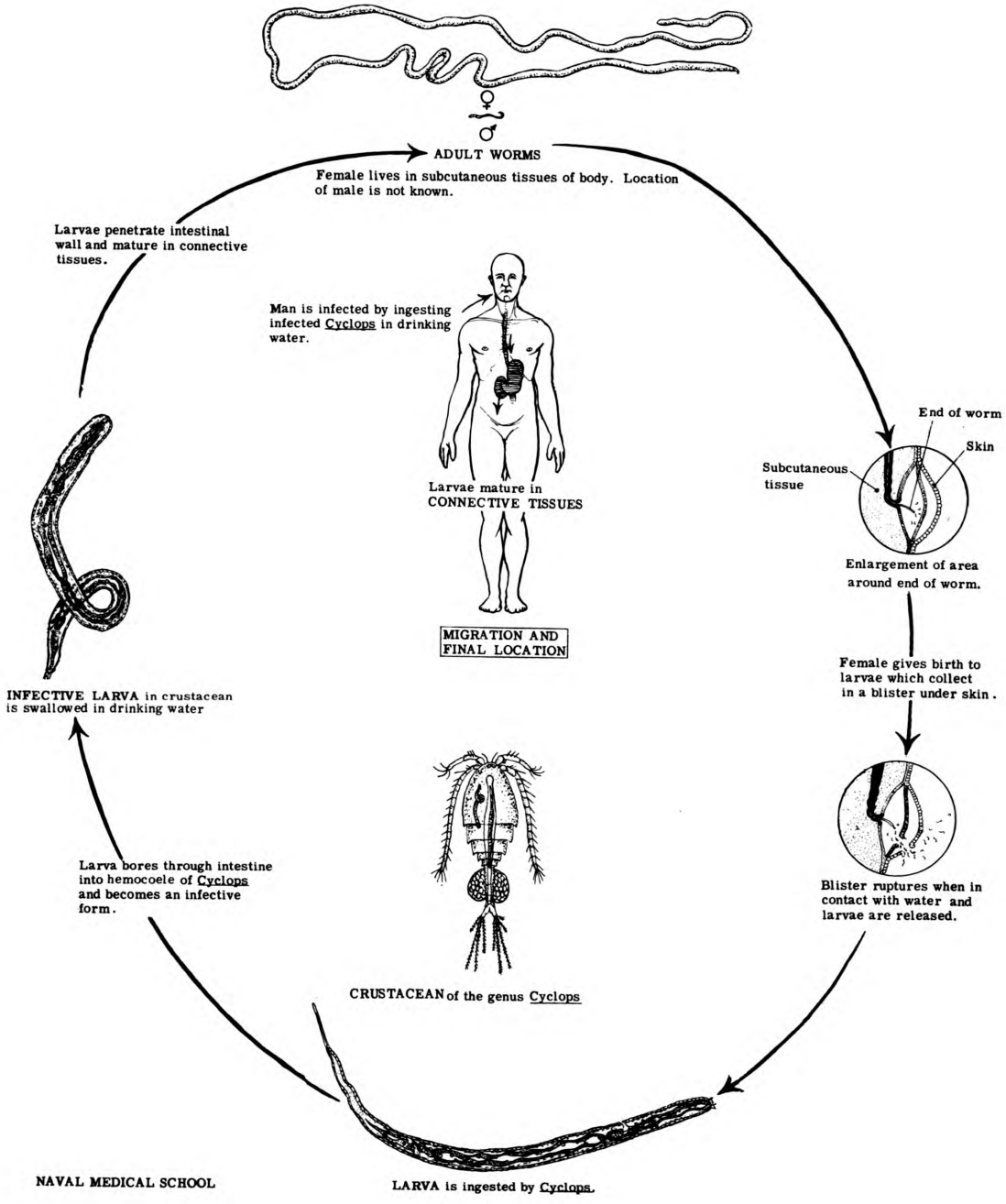


Fig. 79

mouth is surrounded by a shield and eight papillae. The gravid female is about 1 meter long and 1.5 mm. in diameter. The caudal end is sharply curved and probably serves as an anchoring hook. The vulva is situated close to the head end. The uterus of the gravid female is distended with larvae and occupies most of the worm. A number of males have been obtained from experimentally infected dogs. They are mere midgets as compared with the female, the average length being about 25 mm. The posterior end is curled and carries two unequal spicules and a gubernaculum.

Larvae - The larvae are rhabditiform with a posterior esophageal bulb. They average about 600 microns in length, are slightly flattened, have a long slender tail, and are thus well adapted for swimming.

Life Cycle (Fig. 79)

Man is not the only definitive host for this parasite; it has been found in dogs, cattle, and monkeys in the Old World and in foxes, raccoons, minks, and other animals in North America. The adults develop in the body cavities and other internal locations. Only a single male has been found in man. The females are usually first seen when they migrate to the subcutaneous tissues and blister the skin to discharge the larvae. These do not escape into the tissues, as is the case with filariae, but are expelled through the blister or ulcer produced by the worm. The larvae are designed for a free-living existence in water and are normally discharged only when the exposed end of the worm or the blister comes in contact with water. The larvae must be ingested by the intermediate host, Cyclops. Infective forms develop in the body cavity of Cyclops, metamorphosis requiring about 10 days. The host is infected when the crustaceans are ingested.

LABORATORY DIAGNOSIS OF TISSUE ROUNDWORM INFECTIONS

The diagnosis of filarial disease is usually made by finding and identifying the characteristic microfilariae; adults are rarely recovered. The microfilariae of all species except Onchocerca occur most commonly in the blood. Those of Wuchereria bancrofti and W. malayi may also be found in fluid aspirated from a hydrocele or an enlarged lymph node and rarely in the urine. Onchocerca microfilariae may be found in fluid aspirated from a nodule and in biopsied skin teased out in saline; they are rarely demonstrable in blood.

Living microfilariae may be easily seen in fresh blood smears examined by the low power lens. They are continuously lashing and writhing, moving the red blood cells. Species identification is practically never possible on living unstained embryos; thick blood films as for malaria or concentration methods should be used. Preparations may be stained by Giemsa's methylene blue-eosin or hematoxylin. Although more time consuming and requiring more materials, the latter method results in slides which are more permanent and show the diagnostic features more clearly.

It must be emphasized that the microfilariae of Wuchereria bancrofti and W. malayi do not occur in early cases and may not be found in very late cases. It has rarely been possible to demonstrate circulating forms in Americans with early symptoms of filariasis; it is not expected that these cases will ever progress to the point where microfilariae will occur in more than rare instances. Almost all of the morphological characters used in differentiation of the species are shown (Fig. 80).

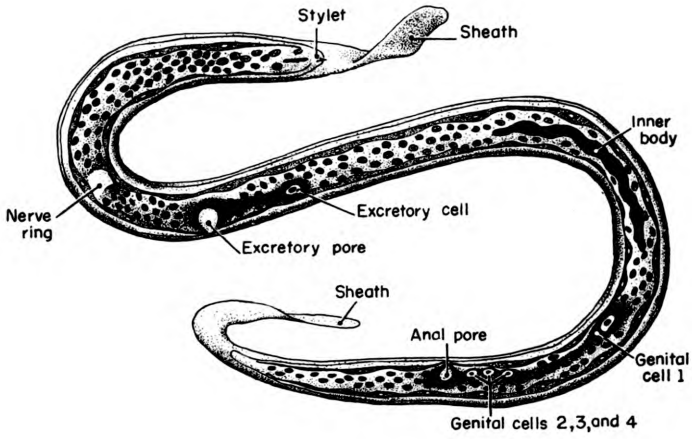


Fig. 80 - Diagram of a microfilaria

MICROFILARIAE

Species	Length (in microns)	Appearance (in stained smears)	Sheath	Stylets	Body Nuclei in Tail
W. bancrofti	230-300	Smooth, sweeping coils	Present	1	Do not extend to tip
W. malayi	175-250	Irregular, kinky coils	Present	2	Extend to tip; last two swollen
L. loa	250-300	Irregular, kinky coils	Present	1	Extend to tip
M. ozzardi	185-200	Smooth curves	Absent	1	Do not extend to tip
A. perstans	190-200	Smooth curves	Absent	None	Extend to tip
O. volvulus	150-285 or 285-370	Smooth, sweeping coils	Absent	None	Do not extend to tip

If the species cannot be identified by the criteria previously given (page 170), other features may be used; however, the clarity of morphological landmarks depends upon good fixation and staining, and if these characters are indistinguishable, it is unlikely that other anatomical features will be seen. Some of the more helpful points of microfilarial behavior and additional diagnostic characters are briefly discussed.

Periodicity - As mentioned before, the microfilariae of W. bancrofti may show a marked or modified nocturnal periodicity, or even a diurnal rhythm, depending upon the geographical area involved; W. malayi usually shows a moderately marked nocturnal periodicity. Loa loa has a sharply marked diurnal rhythm and the other species no periodicity whatsoever. Blood should be taken for examination accordingly.

Anterior end of microfilariae - This is of importance in Loa loa which has a more or less flattened head, the other species being rounded. The point at which the nuclei terminate in the cephalic end is also of help in distinguishing Wuchereria bancrofti from W. malayi: In the former the space without nuclei is about equal in length and width; in the latter it is about twice as long as wide.

Tip of tail - The shape of the tail may be helpful in differentiation. It is usually straight and tapers to a point in W. bancrofti, sharply pointed with a swollen tip in W. malayi, usually recurved in L. loa, sharply pointed in O. volvulus and M. ozzardi, and bluntly pointed in A. perstans.

Anatomical structures - In the column of body nuclei seen after staining, there are certain definite nonnucleated or clear areas which represent regions where various organs will be formed. These are the nerve ring, the excretory pore or "V" spot, and the anal pore or tail spot.

The nucleus of the excretory cell and the four nuclei of the so-called genital cells are more prominent than others. Although these latter cells have usually been considered to be the primordia of the genital organs, it has been shown that they are more likely to be associated with the rectum and anus. A collection of granules found in the posterior half of the body of some microfilariae is called the "Binnen Korper" or inner body. These features are more or less constant in position in a given species and are usually expressed as percentages of the total body length, measuring the distance to them from the anterior end. Certain precautions should be understood before placing too much dependence upon these landmarks: It is extremely difficult for even the expert to measure these distances accurately to a fraction; only faultlessly fixed and stained specimens are satisfactory for study.

	W. bancrofti	W. malayi	L. loa
Nerve Ring	20	26	20
"V" Spot	29	35	30
Tail Spot	83	90	84
Excretory Cell	31	36	36
First Genital Cell	70	70	71

Reaction to stain - Anatomical features are more easily stained in some species than in others. Thus W. malayi is much more difficult to stain well than is W. bancrofti, and dilute Giemsa's which brings out the sheath of the latter fails to stain this structure in Loa loa. The reaction of living microfilariae to methylene blue is sometimes used to separate species, especially W. bancrofti and L. loa; the former absorbs the stain while the latter does not.

Adult worms obtained by biopsy are almost always females and are difficult to identify (see key, page 188). The cuticular markings and the structure of the anterior and posterior ends are the characters most used.

Considerable work has been done recently on skin and complement fixation tests. The reaction is a group one, and antigen prepared from the closely related filaria, Dirofilaria immitis, of the dog may be used in the diagnosis of any one of these filarial diseases. This antigen has been used especially for bancroftian infections in American service personnel in the early stage of the disease. Various workers have found the intradermal test to give approximately 90% or more positive results with very few or no false positives.

The laboratory diagnosis of infection with Dracunculus medinensis is best made by recovering larvae from the blister formed in the skin of the host. Immersion of the part in water will stimulate the discharge of the embryos. Serological tests are available but are not of much value.

Roentgen ray evidence of calcified adult filarial worms or the Guinea worm in the subcutaneous tissues can be obtained in some cases.

KEYS FOR IDENTIFYING EGGS, LARVAE, MICROFILARIAE, AND ADULTS OF THE HELMINTHS

KEY TO THE EGGS OF HELMINTHS

1. Ovum with an operculum, sometimes inconspicuous 2
   Ovum nonoperculate. 11
2. Ovum small, less than 35μ, containing a developed larva when passed 3
   Ovum large, over 50μ, without developed larva when passed 6
3. Organs of larva, particularly the cytolytic glands, asymmetrical 4
   Organs of larva symmetrically arranged. 5
4. Ovum with pronounced shoulders; bulb-shaped; size, 27 to 35μ x 12 to 20μ; in feces or from duodenal drainage . . . *Clonorchis sinensis*
   Ovum with slight shoulders; not bulb-shaped, but more slender, tapering toward each pole; size, 30 x 12μ; in feces or from duodenal drainage . . . . . *Opisthorchis felineus*
5. Ovum widest below middle; size, 26 to 28μ x 15 to 17μ; in feces . . . . . *Metagonimus yokogawai*
   Ovum widest at middle; size, 20 to 30μ x 15 to 17μ; in feces. . . . . *Heterophyes heterophyes*
6. Ovum ovoid in shape 7
   Ovum rhomboidal, broadest in middle and tapering toward both poles; size, 160 to 170μ x 60 to 70μ; in feces. . . . . *Gastrodiscoides hominis*
7. Ovum very large, over 130μ in length 8
   Ovum medium-sized, less than 120μ in length 9
8. Yolk granules evenly distributed throughout yolk cells; size, 130 to 140μ x 80 to 90μ; in feces . . . . . *Fasciolopsis buski*
   Yolk granules concentrated around nuclei of yolk cells; size, 130 to 150μ x 65 to 90μ; in feces or from duodenal drainage . . . . . *Fasciola hepatica*
9. Operculum very distinct and flattened with definite shoulders; dark golden brown in color; size, 75 to 120μ x 45 to 65μ; in sputum; in feces in one-third to one-half of the cases. . . . . *Paragonimus westermani*
   Operculum not flattened, often indistinct; yellowish-brown in color; in feces 10
10. Relatively thick-shelled; broadly barrel-shaped with broad operculum; size, 55 to 75μ x 40 to 55μ . . . . . *Diphyllobothrium latum*
    Relatively thin-shelled; oval in shape with narrow operculum; size, 85 to 115μ x 55 to 70μ . . . . . *Echinostoma ilocanum*
11. Ovum contains a ciliated larva; with conspicuous spine or minute knob. 12
    Ovum does not contain a ciliated larva; without a spine or minute knob 14
12. Ovum with a conspicuous spine 13
    Ovum with a minute knob (frequently difficult to demonstrate); size, 70 to 105μ x 55 to 80μ; in feces. . . . . *Schistosoma japonicum*

Differential Characteristics of

IMPORTANT HUMAN FLUKES

EGGS

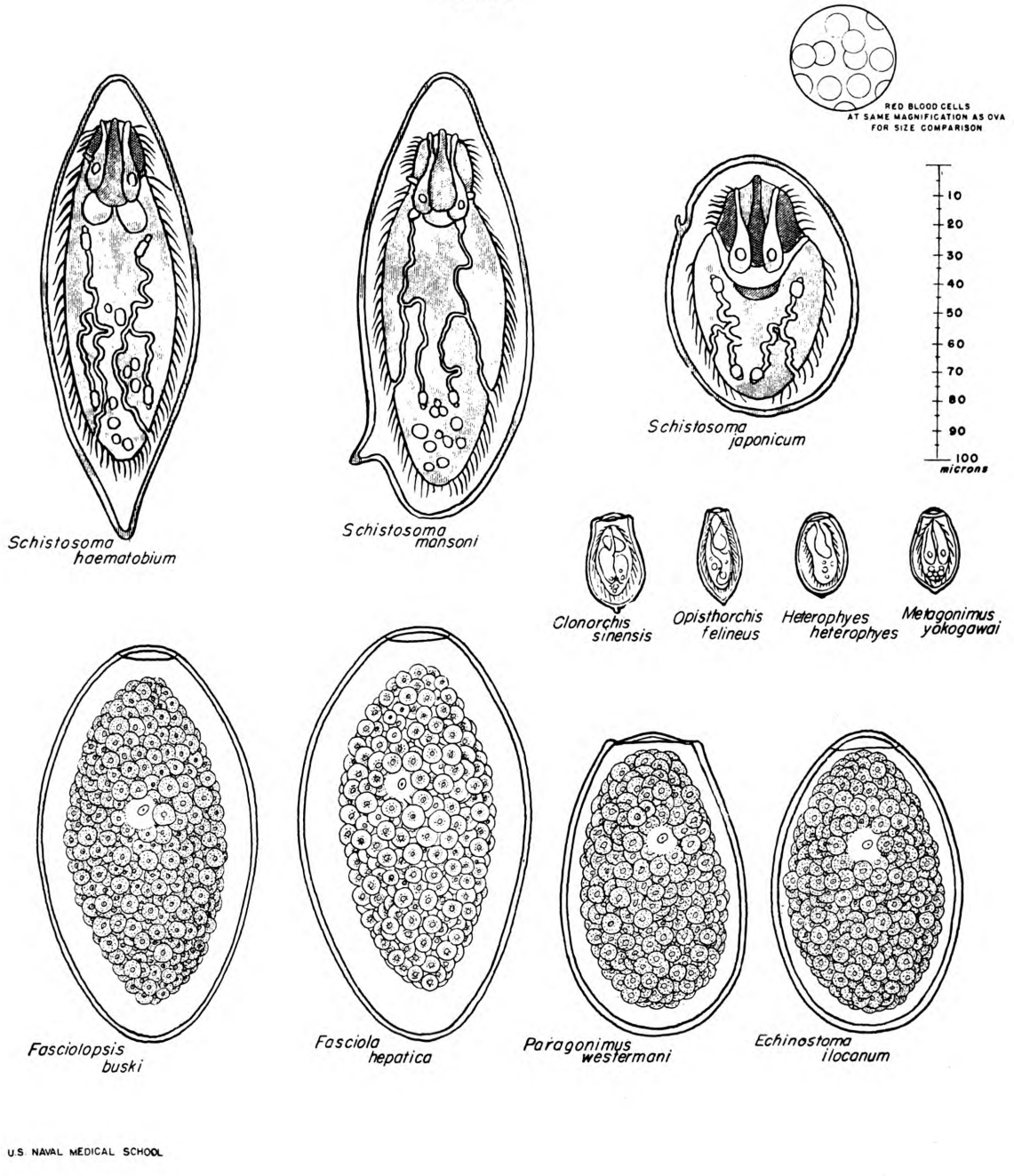


Fig. 81

13. Ovum with terminal spine; size, 110 to 170μ x 40 to 75μ; in urine, rarely in feces . . . . . Schistosoma haematobium
    Ovum with lateral spine; size, 110 to 180μ x 45 to 75μ; in feces or biopsy of rectal ampullae, rarely in urine . . . . . Schistosoma mansoni
14. Ovum contains an embryo with three pairs of hooklets. 15
    Ovum embryonated or unembryonated but never with hooklets 18
15. Ovum with a single thick, brown, radially pitted shell (embryophore); spherical (diameter, 30 to 50μ) or subspherical (30 to 40μ x 20 to 30μ). . . . . Taenia species
    (must be differentiated by examination of mature proglottids or by other adult characteristics)
    Ovum with outer and inner shell separated by space; outer shell moderately thin without radial pitting; inner shell thin, colorless 16

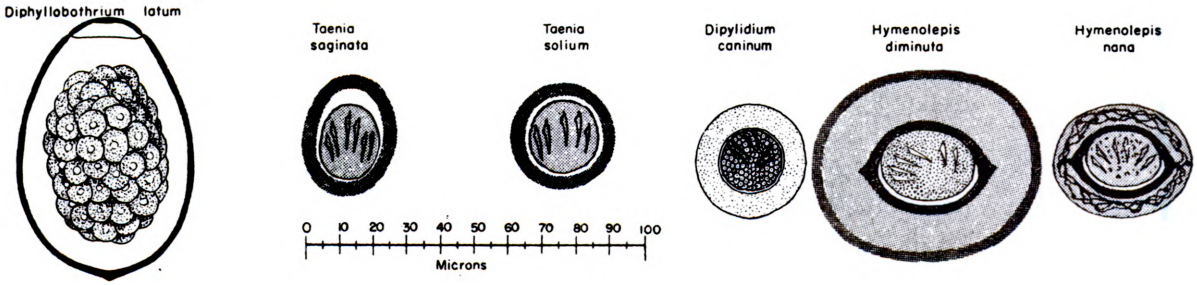


Fig. 82 - Eggs of important human cestodes

16. Ovum single; inner shell with polar thickenings 17
    Ova in packets of usually 10 to 12; inner shell without thickenings; outer shell transparent; spherical; diameter, 20 to 50μ. . . . . Dipylidium caninum
17. Outer shell transparent; inner shell with polar filaments; diameter, 30 to 60μ . . . . . Hymenolepis nana
    Outer shell light yellowish-brown; inner shell without polar filaments; size, 70 to 85μ x 60 to 80μ . . . . . Hymenolepis diminuta
18. Ovum with dark brown shell. 19
    Ovum with clear transparent shell 22
19. Ovum barrel-shaped; with mucoid plug at each pole; with smooth shell 20
    Ovum oval or ovoid, without mucoid plugs at the poles; with rough mammillated shell. 21
20. Shell with radial striations; size, 50 to 65μ x 30 to 35μ; rare, spurious parasite . . . . . Capillaria hepatica
    Shell without radial striations; size, 50 to 54μ x 22 to 23μ . . . . . Trichuris trichiura

Differential Characteristics of

IMPORTANT HUMAN ROUNDWORMS

EGGS

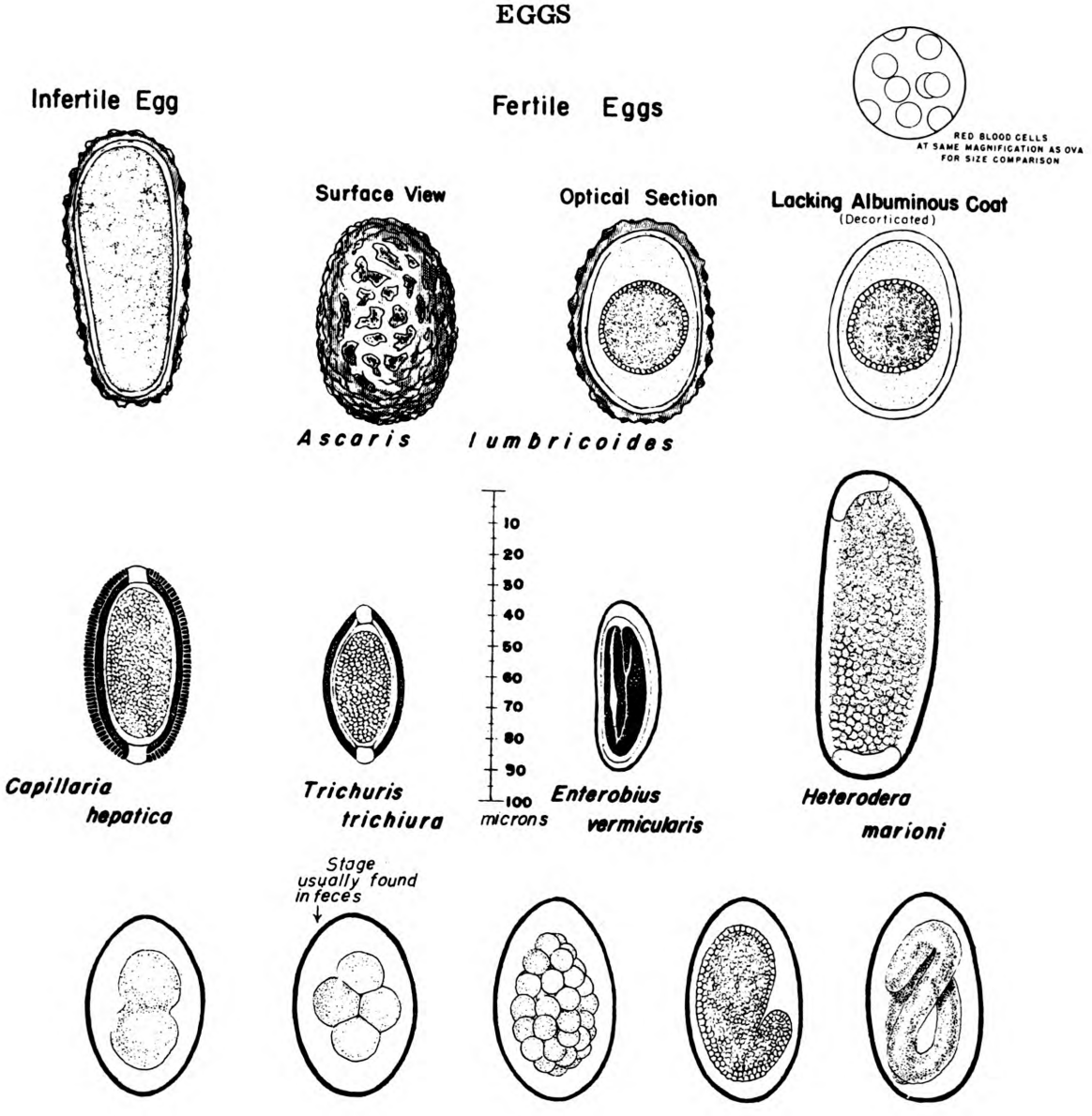


Fig. 83

21. Ovum broadly oval; size, 45 to 75μ x 35 to 50μ . . . . . Ascaris lumbricoides (fertile)
Ovum elongate, oval, or rhomboidal; size, 88 to 95μ x 40 to 45μ . . . . . Ascaris lumbricoides (infertile)
22. Shell very thick; rare 23
Shell thin; much more common forms 24
23. Contents not segmented; size, 43 to 68μ x 33 to 48μ . . . . . Ascaris lumbricoides (decorticated)
Developed to larva stage; size, 50 x 35μ . . . . . Physaloptera caucasica
24. Ovum contains a developed rhabditoid larva 25
Ovum contains embryos in various stages of development but not beyond morula stage. 27
25. Ovum flattened on one side; size, 50 to 60μ x 20 to 30μ . . . . . Enterobius vermicularis
Ovum not flattened on one side, oval 26
26. Rare, only in diarrheal stools; size, 50 to 58μ x 30 to 34μ . . . . . Strongyloides stercoralis
Rare, only in old or constipated stools; size, 56 to 76μ x 35 to 70μ; (must be differentiated by adults or filariform larvae). . . . . Hookworm species
27. Ovum bean-shaped with air spaces at poles; developed to morula stage; size, 80 to 120μ x 25 to 45μ; rare, spurious parasites . . . . . Heterodera marioni
Ovum oval. 28
28. Both poles of egg broadly rounded 29
One pole of egg more pointed than other; embryo usually developed to morula stage; size, 70 to 105μ x 35 to 55μ . . . . . Trichostrongylus species
29. Embryo usually developed to four-celled stage; size, 56 to 76μ x 34 to 50μ; common . . . . Ancylostoma species, Necator americanus (must be differentiated by adults or filariform larvae)
Embryo usually developed beyond four-celled stage; size, 80 x 50μ; rare . . . . . Ternidens deminutus

KEY TO THE LARVAL FORMS OF HELMINTHS

1. Larval form a nematode, a thread-like cylindrical worm most commonly recovered from feces, blood, or sputum, in only a few cases from tissues 2
Larval form a cestode, a round, oval, or amorphous body usually with a typical tapeworm scolex, often showing hooks and suckers; almost always recovered by tissue biopsy, rarely from sputum, never from feces or blood. 19
2. Larvae - forms in which some of the internal organs, usually the intestine, can be demonstrated 3
Microfilariae - forms in which the internal organs are in an embryonic state of development, being represented by configurations of body nuclei. 14

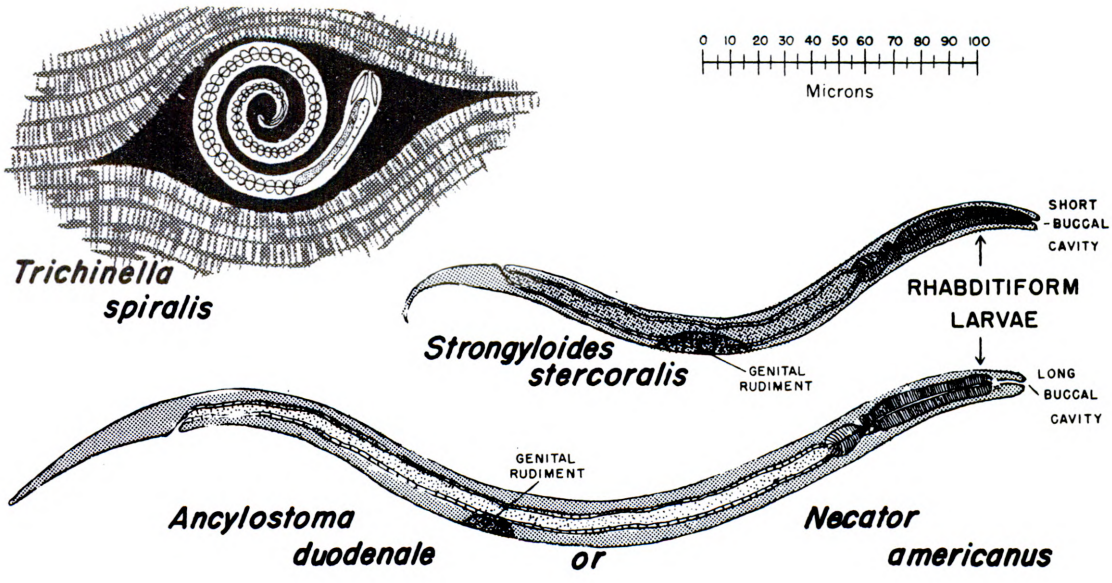


Fig. 84 - Larvae of important human nematodes

3. Larvae recovered from feces or by fecal culture. 4
   Larvae recovered from other excreta or from tissue 11
4. Esophagus rhabditiform or pseudorhabditiform in type. 5
   Esophagus filariform or pseudofilariform in type 8
5. Tail short, ends in a point. 6
   Tail long, filamentous, usually ending in a minute knob. . . . . .Trichostrongylus species
6. Esophagus with only a posterior muscular bulb; genital primordium large or small 7
   Esophagus with a median and posterior muscular bulb; genital primordium small, about 20μ in length; size, 0.24 to 0.3 mm. . . . . .Rhabditis species
7. Buccal cavity short, about equal to body width in length; genital primordium large, about 35μ in length; size, 0.2 to 0.25 mm. . . . . Strongyloides stercoralis
   Buccal cavity longer than body is wide; genital primordium small, about 20μ in length; size, 0.25 to 0.3 mm. . . . . Ancylostoma species, Necator americanus (must be differentiated by filariform larvae or adults)
8. Tail smooth 9
   Tail tuberculated. . . . . .Trichostrongylus species

9. Tip of tail ends in sharp point; genital primordium near middle of body 10
   Tip of tail minutely notched; genital primordium behind middle of body; size, 0.5 to 0.7 mm. . . . . .Strongyloides stercoralis

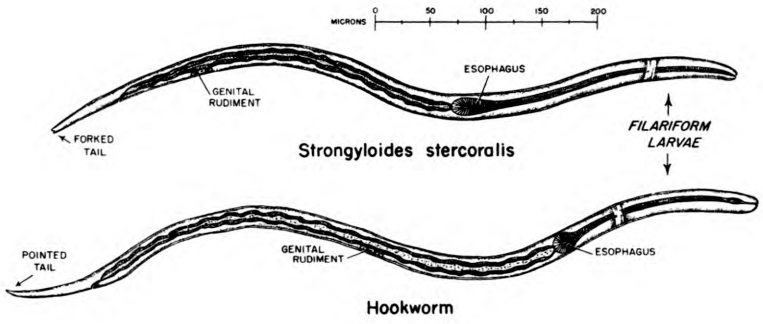


Fig. 85 - Filariform larvae of important human nematodes

10. Head slightly flattened; without definite constriction at the esophageal-intestinal junction; esophageal spears unequal and inconspicuous; size, 0.6 to 0.8 mm. . . . . .Ancylostoma duodenale
    Head not flattened; with marked constriction at esophageal-intestinal junction; esophageal spears equal and conspicuous; size, 0.6 to 0.8 mm. . . . . .Necator americanus
11. Larvae recovered from sputum. 12
    Larvae recovered from spinal fluid, blood, or tissue 13
12. Esophagus rhabditiform in type; size, 0.3 to 2 mm. . . . . Strongyloides stercoralis, Ascaris lumbricoides (See couplet 7 for differentiation of species)
    Esophagus filariform in type; size, about 0.7 mm. . . . Ancylostoma species Necator americanus, Strongyloides stercoralis (rare) (See couplet 9 for differentiation of species)
13. Larvae recovered by muscle biopsy (size, 0.1 to 1 mm.) or rarely from blood or spinal fluid (size, about 0.1 mm.) . . . . . .Trichinella spiralis
    Larvae recovered from fluid taken from skin blister; size, about 0.6 mm. . . . . Dracunculus medinensis
14. Microfilariae in blood, rarely in fluid aspirated from a hydrocele or lymph gland 15
    Microfilariae in fluid aspirated from a skin nodule, or teased out from a skin snip; size, 150 to 370μ . . . . . Onchocerca volvulus
15. Microfilariae with sheath 16
    Microfilariae lacking sheath 18
16. Body nuclei do not extend to tip of tail; one stylet; lies in smooth sweeping curves; size, 230 to 300μ . . . . . .Wuchereria bancrofti
    Body nuclei extend to tip of tail; lies in irregular kinky coils 17

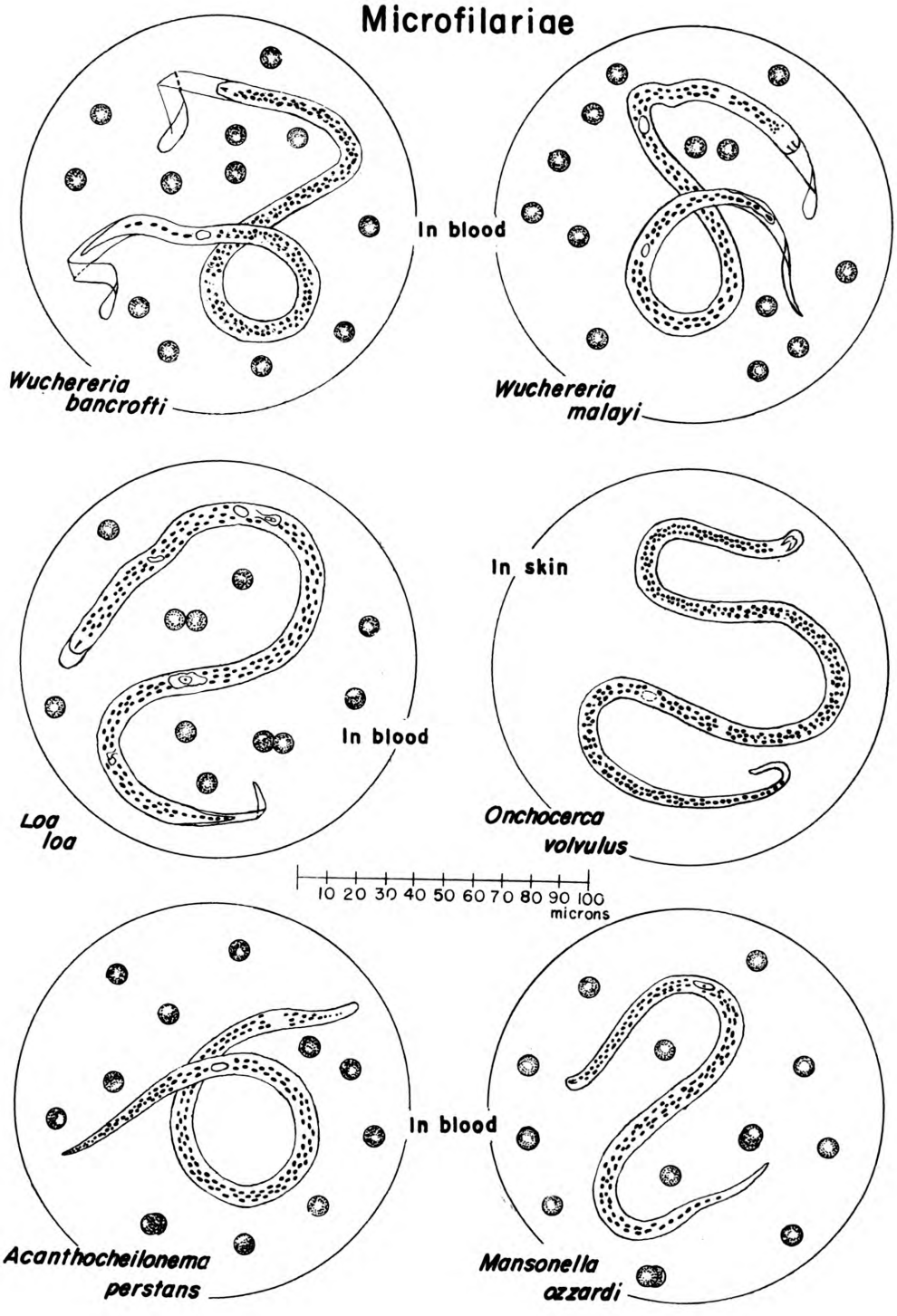

Microfilariae
In blood
Wuchereria bancrofti
Wuchereria malayi
In blood
Loa loa
In skin
Onchocerca volvulus
10 20 30 40 50 60 70 80 90 100
microns
In blood
Acanthocheilonema perstans
Mansonella ozzardi

Fig. 86

17. Tip of tail swollen at region of last two nuclei; head not flattened; two stylets; size, 175 to 250μ . . . . . Wuchereria malayi
    Tip of tail tapers to point; head flattened, one stylet; size, 250 to 300μ . . . . . Loa loa
18. Body nuclei extend to tip of tail; size, 190 to 200μ . . . . . Acanthocheilonema perstans
    Body nuclei do not extend to tip of tail; size, 185 to 200μ . . . . . Mansonella ozzardi
19. Larvae with hooklets and suckers; usually round or oval 20
    Larvae with anterior invagination without suckers or hooklets; usually elongated ribbon-like (plerocercoid or sparganum); size, about 3 to 30 mm. . . . . Diphyllobothrium species
20. Larvae with single bladder and scolex armed with hooklets (cysticercus); size, about 10 x 5 mm. . . . . Taenia solium
    Larvae with many bladders and many armed scolices (echinococcus or hydatid); usually single scolices are recovered which are about 150μ long . . . . . Echinococcus granulosus

KEY TO THE ADULT HELMINTHS

1. Without complete alimentary canal - mouth and intestine present or absent, always without anus; body more or less flattened; with hold-fast organs in the form of grooves or suckers 2
   With complete alimentary canal - mouth, intestine, and anus present; body round in cross section; without suckers . . . . Class Nematoda
2. Body of adult not segmented; one or two suckers present; mouth and intestine present; parasitic in liver, lungs, intestine, occasionally elsewhere . . . . . Class Trematoda
   Body of adult segmented; with two sucking grooves or four suckers; alimentary tract absent; adults parasitic in intestine. . . . . Class Cestoidea

Class Trematoda

1. Sexes separate (dioecious) 2
   Sexes united (monoecious or hermaphroditic) 4
2. Male with finely tuberculated or smooth integument; female with large number of eggs in uterus 3
   Male with grossly tuberculated integument; female with not more than four eggs in uterus. . . . . Schistosoma mansoni

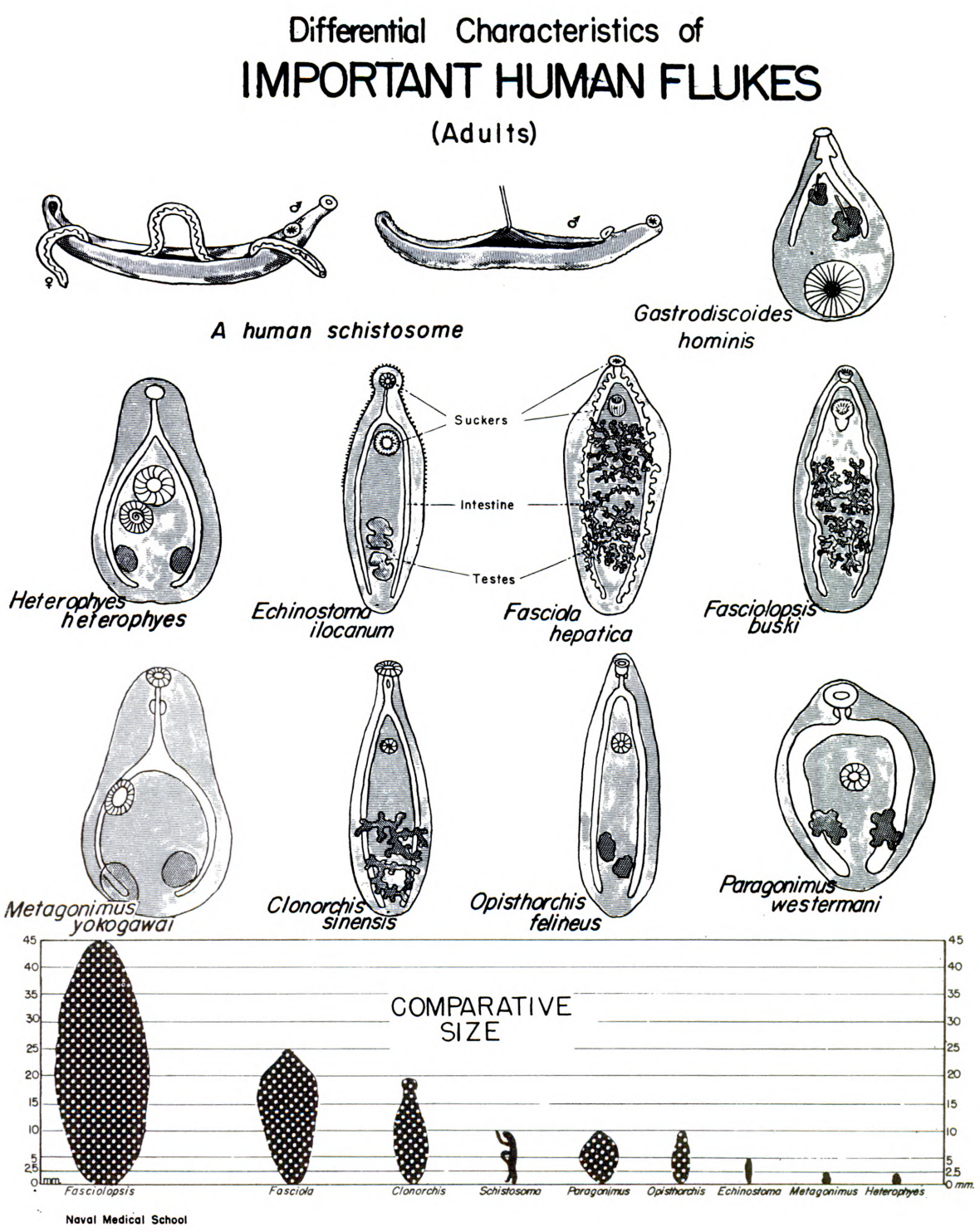

Differential Characteristics of
IMPORTANT HUMAN FLUKES
(Adults)
♀
♂
A human schistosome
Gastrodiscoides hominis
Suckers
Intestine
Testes
Heterophyes heterophyes
Echinostoma ilocanum
Fascida hepatica
Fasciolopsis buski
Metagonimus yokogawai
Clonorchis sinensis
Opisthorchis felineus
Paragonimus westermani
COMPARATIVE SIZE
45
40
35
30
25
20
15
10
5
2.5
0 mm.
Fasciolopsis
Fasciola
Clonorchis
Schistosoma
Paragonimus
Opisthorchis
Echinostoma
Metagonimus
Heterophyes
Naval Medical School

Fig. 87

3. Male with four testes; egg from uterus of female showing terminal spine . . . . . Schistosoma haematobium
   Male with seven testes; egg from uterus of female with rudimentary lateral spine . . . . . Schistosoma japonicum
4. Ventral sucker usually anterior to middle of body, never at posterior end 5
   Ventral sucker conspicuous, at or near posterior end of body . . . . . Gastrodiscoides hominis
5. With only an oral and ventral sucker 6
   With a large genital sucker in addition to the oral and ventral suckers . . . . . Heterophyes heterophyes
6. With a circlet of prominent spines around the oral sucker . . . . . Echinostoma ilocanum
   Without such a circlet of spines 7
7. Intestinal ceca unbranched 8
   Intestinal ceca with secondary branchlets . . . . . Fasciola hepatica
8. Less than 2 cm. in length; testes lobed or nonlobed but never with fine branches 9
   Two to 7 cm. in length; testes extremely branched . . . . Fasciolopsis buski
9. Testes lobed. 10
   Testes not lobed. . . . . Metagonimus yokogawai
10. Testes with few primary lobes 11
    Testes with many secondary lobes . . . . . Clonorchis sinensis
11. Testes deeply lobed . . . . . Paragonimus westermani
    Testes weakly lobed. . . . . Opisthorchis felineus

Class Cestoidea

1. Scolex or "head" recovered 2
   Gravid proglottis recovered 7
2. Scolex with two longitudinal, slit-like grooves or suckers; about 1 mm. in diameter . . . . . Diphyllobothrium latum
   Scolex with four suckers 3
3. Scolex with rostellum armed with hooks 4
   Scolex unarmed 6
4. Scolex globular, large, about 1 mm. in diameter, armed with two rows of hooks . . . . . Taenia solium
   Scolex club-shaped, small, less than 0.5 mm. in diameter 5
5. Scolex armed with one row of hooks; about 0.3 mm. in diameter . . . . . Hymenolepis nana
   Scolex armed with three to seven circlets of hooks; 0.25 to 0.5 mm. in diameter. . . . . Dipylidium caninum
6. Scolex pear-shaped, large, 1 to 2 mm. in diameter . . . . . Taenia saginata
   Scolex club-shaped, small, about 0.5 mm. in diameter . . . . . Hymenolepis diminuta

7. Segment longer than broad. 10
   Segment broader than long. 8
8. Genital pores ventral; uterus rosette-shaped . . . . . Diphyllobothrium latum
   Genital pores lateral; uterus a transverse elongated sac. 9
9. Segments less than 1 mm. in breadth . . . . . Hymenolepis nana
   Segments about 2.5 mm. in breadth . . . . . Hymenolepis diminuta
10. Uterus with median stem and a varying number of lateral branches; with single genital pore 11
    Uterus composed of a large number of pockets, each filled with ova; genital pores double . . . . . Dipylidium caninum
11. Uterus with 5 to 13 main lateral branches on each side . . . . . Taenia solium
    Uterus with 15 to 30 main lateral branches on each side . . . . Taenia saginata

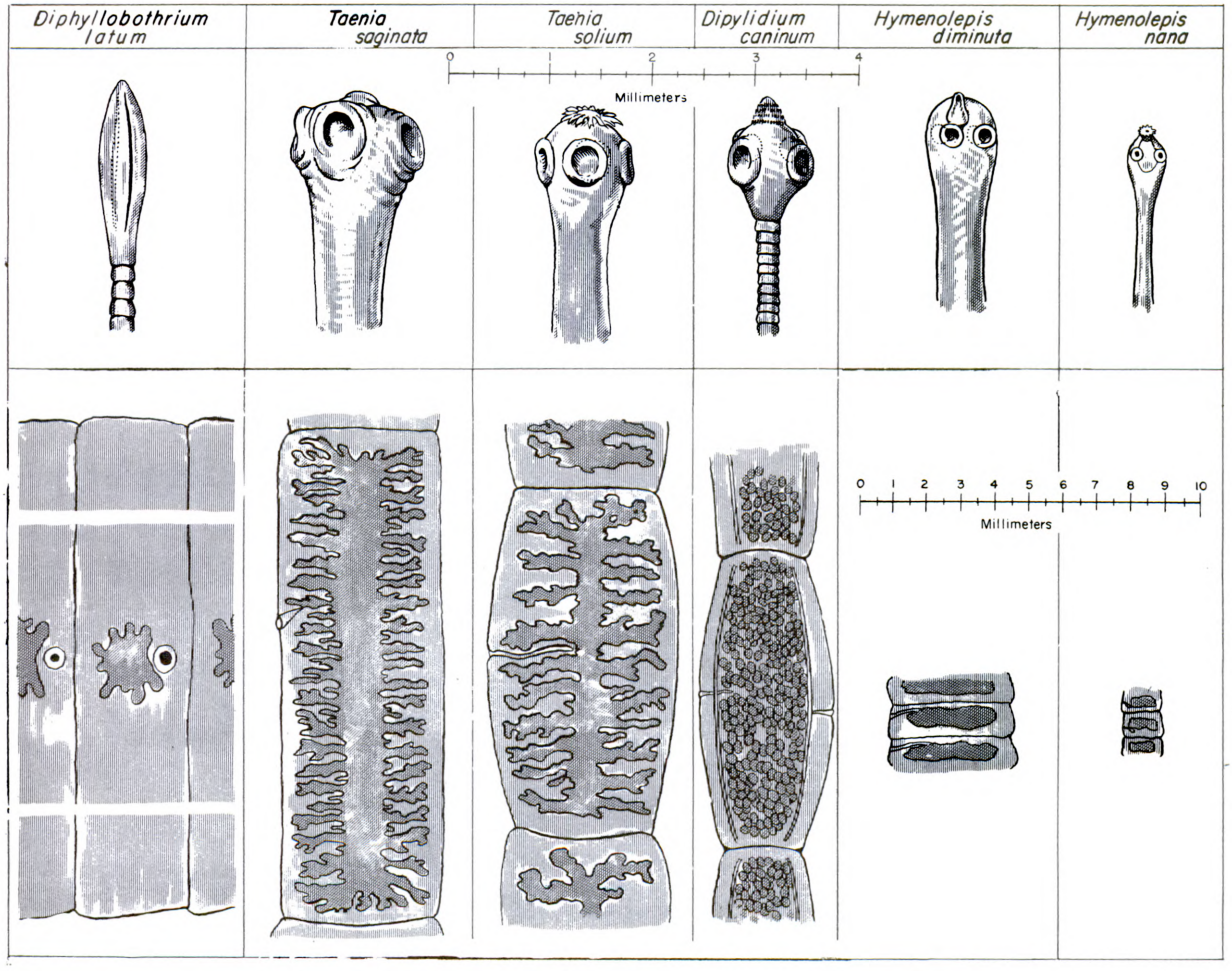


Fig. 88 - Adults of important human cestodes

Adult Helminths

Class Nematoda

1. Worms with different regions of the body varying in diameter, usually tapering toward the extremities; usually found in the alimentary tract or embedded in the intestinal mucosa 2
   Worms long, slender, and thread-like throughout entire length; found in tissues of man, never in the intestine 13
2. Worms with a more or less slender, thread-like anterior region and a thicker posterior portion; esophagus inconspicuous, with long posterior region composed of a small tube surrounded by a single row of glandular cells 3
   Worms tapering to either end but never as above; esophagus prominent, with heavily muscled posterior region and triradiate lumen. 4
3. Large worms (female, 35 to 50 mm.; male, 30 to 45 mm.) with anterior end of body long and whip-like . . . . . *Trichuris trichiura*
   Small worms (female, 3 to 4 mm.; male, 1.4 to 1.6 mm.) with anterior end filiform but not whip-like . . . . . *Trichinella spiralis*
4. Anterior extremity markedly bent, males with a conspicuously widened copulatory bursa 5
   Anterior extremity straight or slightly curved, males with or without a copulatory bursa 8
5. Mouth armed with hook-like teeth. 6
   Mouth armed with cutting plates (female, 9 to 11 mm.; male, 5 to 9 mm.). . . . . *Necator americanus*
6. Two pairs of hook-like teeth 7
   Three well-developed pairs of hook-like teeth, the outermost the largest and the innermost the smallest (female, 14 mm.; male, 10 mm.) . . . . . *Ancylostoma caninum*
7. Outer pair of teeth large; smaller inner pair without accessory processes (female, 9 to 11 mm.; male, 8 to 8.5 mm.) . . . . . *Ancylostoma braziliense*
   Teeth of equal size, the inner pair with a small accessory process (female, 10 to 13 mm.; male, 8 to 11 mm.) . . . . . *Ancylostoma duodenale*
8. Small worms, less than 15 mm. in length. 9
   Large worms, more than 100 mm. in length (female, 200 to 350 mm.; male, 150 to 310 mm.); male with curved posterior end . . . . . *Ascaris lumbricoides*
9. Anterior end of worm with conspicuous cervical alae; male without bursa copulatrix (female, 8 to 13 mm.; male, 2 to 5 mm.) . . . . . *Enterobius vermicularis*
   Anterior end of worm without cervical alae, male with or without bursa copulatrix. 10
10. Very small worms, less than 2.5 mm. in length; male without bursa copulatrix (female, 1.8 to 2.2; male, 0.5 to 0.8 mm.) . . . . . *Strongyloides stercoralis*
    Medium-sized worms, more than 3.5 mm. in length; male with bursa copulatrix 11

Differential Characteristics of
IMPORTANT HUMAN ROUNDWORMS

Adults Living in Intestine

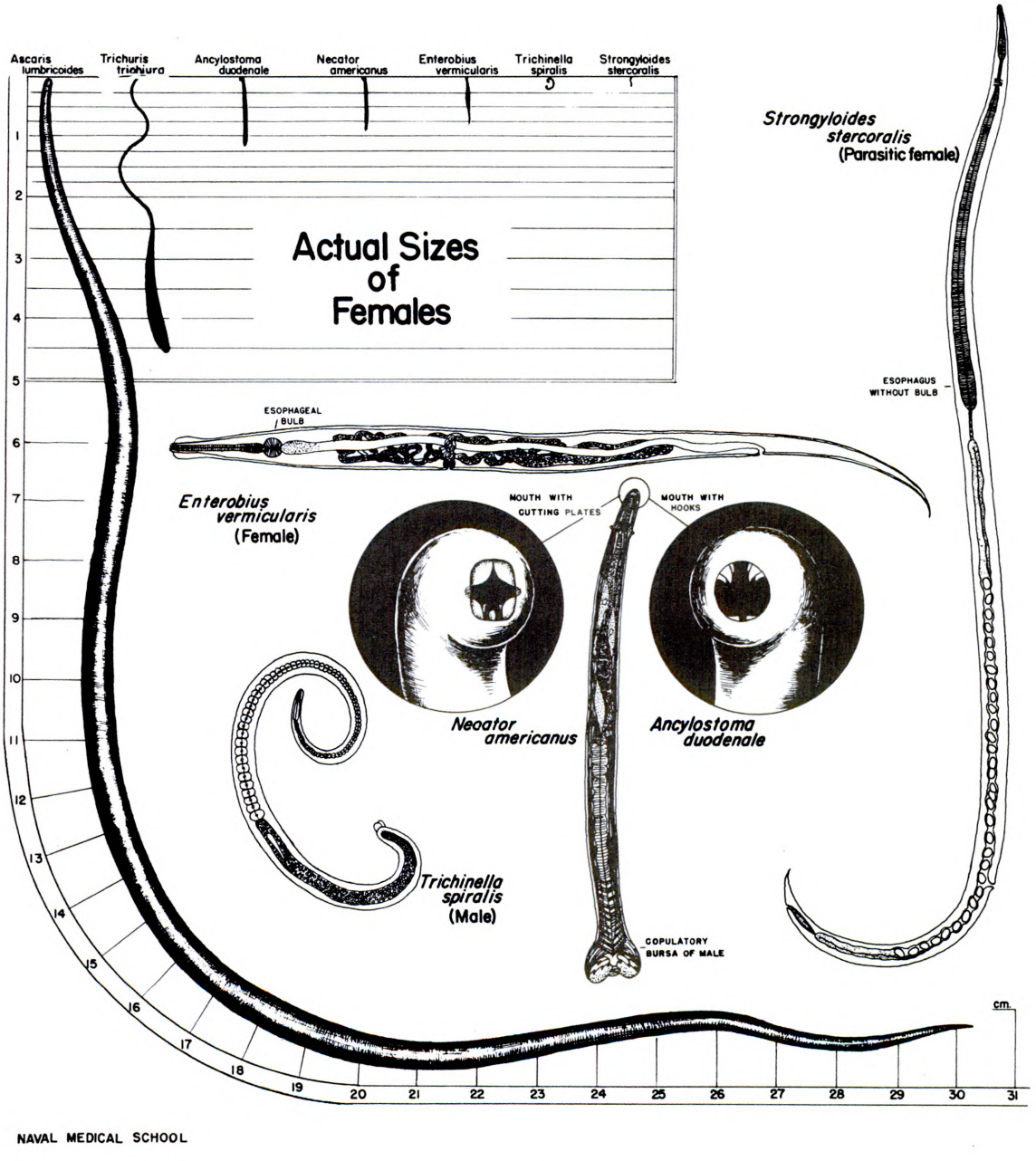


Fig. 89

11. Anterior end blunt; with mouth guarded by a circle of stout bristles (female, 12 to 16 mm.; male, 9.5 mm.) . . . . Ternidens deminutus
    Anterior end tapering to point; without distinct buccal capsule 12
12. Spicules of male barbed (female, 5 to 7 mm.; male, 4 to 5 mm.) . . . . . Trichostrongylus orientalis
    Spicules of male pointed (female, 5 to 6 mm.; male, 4 to 5 mm.) . . . . . Trichostrongylus colubriformis
13. Cuticle with raised nodules or striations 14
    Cuticle smooth. 15
14. Cuticle with nodules or minute bosses (female, 50 to 70 mm.; male, 30 to 35 mm.) . . . . . Loa loa
    Cuticle with annular striations (female, 115 to 700 mm.; male 20 to 40 mm.) . . . . . Onchocerca volvulus
15. Females very long (500 to 1,200 mm.); males rarely found, deep in tissues (12 to 29 mm.) . . . . . Dracunculus medinensis
    Females less than 120 mm. in length; males rarely found 16
16. Tail of female bluntly rounded 17
    Tail of female with appendages 18
17. Tail of male ends in a sharp curve (female, 80 to 100 mm.; male, 40 mm.) . . . . . Wuchereria bancrofti
    Tail of male ends in about three spiral coils (female, 55 mm.; male, 22 to 23 mm.) . . . . . Wuchereria malayi
18. Tail of female bifurcated; head with cuticular shield (female, 70 to 80 mm.; male, 45 mm.) . . . . . Acanthocheilonema perstans
    Tail of female with two fleshy appendages; head unarmed (female, 65 to 80 mm.; size of male not known). . . . . Mansonella ozzardi

LABORATORY METHODS FOR DETECTION AND RECOGNITION OF HELMINTHS

The laboratory methods used to demonstrate the diagnostic stages of helminths are many and diverse. Few or none of them are without disadvantages and only a small number have general adaptability. The laboratory technician must weigh many factors before deciding which technics to use. He must answer such questions as: What are the most common and important helminths in this area? How many specimens will there be per day? How much apparatus is available? How many technicians will be working? Is it important to discover all infections or only the heavier ones? Are quantitative results desired?

Only those procedures which are believed to have practical application have been included here. An attempt has been made to select methods suited to the most primitive conditions as well as to the better equipped laboratories.

Examination of Feces

Feces should be examined for the ova, larvae, or adults of all the flukes (other excreta preferable for S. haematobium and P. westermani), the intestinal tapeworms,

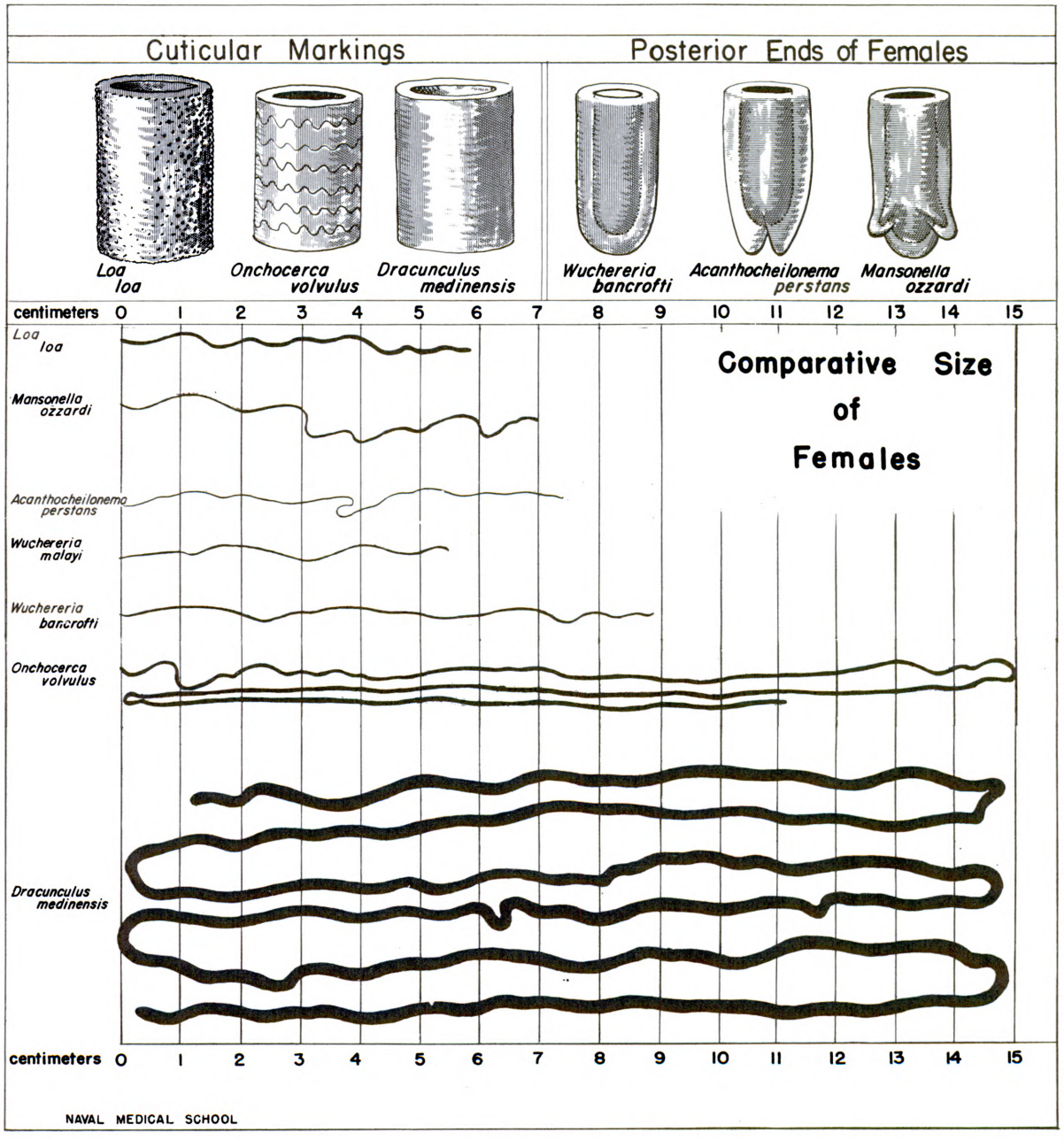

Cuticular Markings
Posterior Ends of Females
Loa loa
Onchocerca volvulus
Dracunculus medinensis
Wuchereria bancrofti
Acanthocheilonema perstans
Mansonella ozzardi
centimeters 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Loa loa
Mansonella ozzardi
Acanthocheilonema perstans
Wuchereria malayi
Wuchereria bancrofti
Onchocerca volvulus
Dracunculus medinensis
Comparative Size of Females
centimeters 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
NAVAL MEDICAL SCHOOL

Fig. 90

and the intestinal roundworms (other methods preferable for Enterobius). In most instances several methods are available, and at least the direct smear and one of the more refined technics should be employed before a negative report is rendered.

Stools should be collected in clean, dry containers. Examination should be made as soon as practical after collection, but in most cases helminthic material will remain identifiable for several days if the stool is kept in a cool environment. Specimens obtained by purging or enemas may be positive when the ordinary stool is negative. Representative fecal portions may be placed in proper size vials containing MIF (see page 54) or 6 per cent formaldehyde as a preservative (1 part of feces to 4-5 parts of preservative) carefully packed to avoid breakage and shipped to diagnostic laboratories.

The ova and larvae of the helminths may be found most efficiently under the low-power (16-mm.) objective. Most workers prefer a 10x ocular and a rather subdued illumination. After the parasites are found, they may be more closely examined by the high-dry (4-mm.) objective; the oil-immersion objective is almost never required for identification. All preparations should be examined systematically beginning at one corner and traversing the preparation back and forth until all fields have been viewed (see diagram, page 21).

Direct Smear

This is used as the first and all-purpose method for any stage of helminth which passes in the feces. At least three smears should be examined from three different portions of the specimen; as a rule the outside of the stool gives the highest percentage of positive findings. The ova of the blood flukes occur most frequently in small clots of blood and mucus. The technic is simple and a very small amount of apparatus is required. However, light infections are frequently missed and the method is not quantitative.

Technic - A small specimen from the stool is thoroughly emulsified in saline or tap water by means of an applicator. Any large particles which would keep the cover glass from settling are removed. The smear should be heavier than that employed in searching for intestinal protozoa; newsprint should be just legible through it. A cover glass is placed on the preparation, air bubbles being avoided by the method described under intestinal protozoa. The smear is examined systematically under the low-power lens of a compound microscope. Nematode larvae which are usually very active in the feces may be quieted and stained for identification by iodine, eosin, or other solutions.

Straining

1. For ova and larvae - This method may be used for all helminth ova and larvae. The organisms remain in the filtrate from which the coarse roughage is removed. This method is of advantage in that much of the material which makes the parasites hard to find and recognize is removed. It is of greatest value when combined with other methods. It is of disadvantage in that there is little or no concentration of the parasites, and in many cases ova are lost by the process. It is time consuming, and there is danger of contamination from a previously employed strainer.

Technic - Approximately 1 gm. of feces is mixed in a small amount of water. This is passed through dampened cheesecloth or wire screen (20- to 120-mesh). Loopfuls of the filtrate are examined for parasites.

2. For adult helminths - This method is used particularly for recovering the smaller intestinal worms. Usually these parasites are only found subsequent to treatment; all stools should be examined for 2 to 3 days after the administration of the anthelminthic. This is the best method for recovering the smaller helminths and tapeworm scolices. It is time consuming, however, and of little importance except to determine whether or not the scolex of a tapeworm has been eliminated.

Technic - The fecal specimen is mixed with enough normal saline to give the consistency of a thin paste. The mixture is strained through a graduated series of wire sieves (6-, 12-, 24-, and 40-mesh). The debris is rinsed and examined over a dark background by means of a hand lens or dissecting microscope using reflected light.

Sedimentation

This method is used especially for the schistosome ova, the operculate eggs of the other flukes, and Diphyllobothrium. It may be used for all helminth ova which occur in the feces. Occasionally Trichinella adults may be found during the first few days of the infection by this method. The ova are not distorted; they are concentrated with a small amount of debris and may be easily preserved. However, the method is time consuming and not quantitative. Effective concentration is not as high as that obtained by some of the other technics, especially those used for roundworm ova.

Technic - The fecal specimen is thoroughly mixed in tap water until its consistency is about that of a thin paste. The amount of water required usually falls between 10 and 20 times the volume of the fecal specimen. Mixing is facilitated if the first water added is slightly warm. The diluted specimen is then placed in a tall glass cylinder or a cone-shaped graduate and allowed to stand. After 1 to 2 hours, the top three-fourths is siphoned or poured off, the cylinder refilled with water, and the contents stirred well and allowed to stand for a second 1- to 2-hour period. The sedimentation process is repeated until the supernatant fluid is almost clear. (Caution: Schistosome ova may hatch if the procedure requires more than 1 hour.) Portions of the sediment are removed by means of a pipet to a glass slide, covered, and examined systematically with the low-power objective. Note: Some workers prefer removing the coarse roughage before the first sedimentation by passing the fecal emulsion through a strainer (30- to 120-mesh). (See straining methods for advantages and disadvantages.)

Centrifugation

1. Tap water method - This is used for all helminth ova and larvae in feces. It is the best all-purpose concentration method. However, it is not as effective or simple as some other methods for specific helminths and is not quantitative. The method requires a centrifuge which may not be available.

Technic - A small amount of feces is mixed thoroughly in 10 to 20 times its volume of water until a thin paste is formed. This is strained (20- to 40-mesh) into a centrifuge tube with rounded bottom and spun 1 to 2 minutes at approximately 2,500 revolutions. The supernatant solution is decanted; the tube is filled with water, its contents mixed thoroughly and centrifuged again. This process is repeated until the supernatant fluid is clear. (Note: For most laboratory diagnoses two spinnings are adequate.) The sediment is removed to a slide and examined.

2. Acid-ether method - This is used especially for the schistosome ova. It gives about a fivefold increase over sedimentation methods in numbers of ova found. Its disadvantages are that it is expensive and lacks general applicability.

Technic - About 1 gm. of feces is thoroughly emulsified in 5 ml. hydrochloric acid (40 ml. concentrated hydrochloric acid diluted to 100 ml.) in a test tube. The emulsion is filtered through two layers of moist gauze into a 15-ml. centrifuge tube. An equal quantity of ether is added; the tube is stoppered and shaken thoroughly. It is then centrifuged for 1 minute at 1,500 revolutions. The debris at the acid-ether junction is loosened by ringing with an applicator stick. The acid and ether are poured off rapidly. The sediment is stirred in a few drops of the remaining solution; a drop is decanted to the slide, covered, and examined systematically under the low-power objective.

Flotation

This method is used especially for the intestinal roundworm ova and for the eggs of certain intestinal tapeworms (Hymenolepis nana and H. diminuta). This is a very simple procedure and gives a high effective concentration. It requires little apparatus and time. Its disadvantages are that it is not quantitative and is only applicable in regions where the flukes and tapeworms are not important. It misses infections of nearly all flukes, Diphyllobothrium, and a considerable percentage of Taenia, Strongyloides, and infertile Ascaris.

Solutions - Any one of the solutions listed below may be used. Sodium chloride (table salt) is the least expensive and very efficient if used correctly.

1. Table salt (crude). Saturated solution NaCl - sp. gr. about 1.20 or 1.21.
2. Table sugar. Saturated solution (2 lbs. sugar, 1,125 ml. water). As a preservative, 10 ml. phenol are added.
3. Calcium chloride. Saturated solution $CaCl_2$.
4. Zinc sulfate. Three hundred thirty-one gm. $ZnSo_4$. $7H_2O$ USP in 1 liter water. Sp. gr. 1.180. (A solution of sp. gr. 1.20 is used for formalin-preserved material.)

Technic - About 1 gm. of feces is thoroughly emulsified in a small amount of one of the above solutions in suitable container. Glass vials 2.5 cm. in diameter and 5.0 cm. tall are very satisfactory. The solution is added until it nearly fills the container. More is carefully added until a slight meniscus forms at the top of the vial. A slide (should cover entire surface area) is placed over the vial in

contact with the meniscus. The covered vial is allowed to stand for 10 minutes to 1 hour (never over 30 minutes for brine). The slide is removed by lifting it straight up; it is inverted and examined systematically by the low-power objective. (Note: Alternative methods employ a cover glass instead of the glass slide over the meniscus or dispense with both slide and cover glass, a surface film being removed for examination by means of a loop.). If desired, the specimen may be strained before the ova are floated. This gives a clearer field, but a certain percentage of the eggs are lost; straining is usually not considered necessary.

Combined Concentration of Both Helminth Ova and Protozoan Cysts

Zinc-sulfate centrifugal flotation - This is one of the better known methods for combined concentration of both protozoan cysts and helminth ova. (See page 58 for the technic.) It is applicable only to ova that will float and consequently has all the disadvantages of the flotation methods. It is inferior to direct flotation for helminths alone and requires more time and apparatus. It is not quantitative. It is recommended for laboratories which desire to diagnose both protozoan and worm infections in one procedure but not for helminths alone.

MIFC* - A modified MIF technic for concentration of protozoa and helminth eggs - This method is helpful since it employs the previously described simple MIF method for preservation and staining of fecal samples. Experience has shown that it is more efficient when used for freshly fixed fecal samples than for vials of material that has been stored for some time. Procedure is as follows after fecal sample has been prepared as described for the MIF technic (page 54):

1. Mix the MIF preserved specimen by shaking vigorously for 5 seconds.
2. Strain this mixture through two layers of wet surgical gauze into a 15 ml. centrifuge tube.
3. Add 4 ml. of ether to the centrifuge tube, insert a rubber stopper and shake vigorously. (If ether remains on top after shaking, add 1 ml. of tap water and reshake). The ether used should be refrigerated to reduce volatilization.
4. Remove stopper and let stand for 2 minutes.
5. Centrifuge for 1 minute at 1600 r.p.m. Four layers will appear in the tube: (a) an ether layer on top, (b) a plug of fecal detritus, (c) an MIF layer, (d) the sediment containing protozoa and helminth eggs on the bottom.
6. Loosen the fecal plug by ringing with an applicator stick.
7. Quickly, but carefully, pour off all but the bottom layer of sediment.
8. Thoroughly mix this sediment, pour a drop on a slide, mount with a cover glass and examine.

The time required to prepare the MIFC specimen for examination is about 4 minutes.

*Blagg, W., et al., 1955. A new concentration technic for the demonstration of protozoa and helminth ova in feces. Am. J. Trop. Med. & Hyg. 4:23-28

Estimation of Worm Burden (ova counting)

These methods are not advised for routine laboratory or field work. Their value lies in research and in studies to determine the worm burden of various populations. Probably the most efficient technic to give quantitative results for hookworm, Ascaris, and Trichuris ova is the direct centrifugal flotation method of Lane (1923)**. Stoll (1923)** and others have also designed various efficient ova-counting methods that can be highly recommended. All of the methods require careful technic and special apparatus. The original papers and their modifications should be carefully studied before the methods are employed. The Baermann technic can also be adapted for ova counting.

Hatching Technic

This is used especially for Schistosoma mansoni and S. japonicum ova (see examination of urine for the application of this method to S. haematobium). The method has a high efficiency in disclosing very light infections. It is a simple procedure requiring but little apparatus. However, it is time consuming, and infertile and degenerate ova are missed.

Technic - The fecal specimen is sedimented. This sediment is diluted with clear water and allowed to stand for 1 to 12 hours. The top few centimeters of water are examined with hand lens for free-swimming miracidia. Caution: Free-swimming protozoans (ciliates) occur in stale water and may be confused with the trematode larvae.

Culture Method

This is used primarily for hookworm and Stongyloides larvae. It is a simple technic which requires little apparatus. It has the disadvantage of being time consuming.

Technic - The fecal specimen is mixed with an equal quantity of powdered animal charcoal or sterile sand. Enough water is added to give a soft consistency. The mixture is placed on a piece of wet filter paper in a Petri dish and covered. The vater of condensation on the underside of the cover is examined at various intervals, or the larvae are recovered by using the Baermann technic.

**For additional information on these and related technics consult section on technics: Faust, E.C. and Russell, P.F., 1957. Clinical Parasitology; 943-995. Faust, E.C., 1955. Animal Agents and Vectors of Human Disease; 575-586.

Killing and Fixation of Adult Helminths for Identification

Any of a number of methods may be employed for killing, fixing and staining helminth parasites for study purposes. Ideally helminths should be relaxed before they are fixed since contracted specimens are difficult or in some cases impossible to identify. Living worms may be placed in normal saline or tap water and allowed to stand for several hours until the parasites become limp, relaxed or fail to respond to probing. This process may be hastened by addition of small quantities of menthol or magnesium sulfate crystals to water in which parasites are bathed.

Nematodes may be dropped directly into steaming 5 per cent formalin for killing and fixation. Cestodes and trematodes should be placed in position desired in a flat bottom glass container prior to addition of, or covering with 5 per cent formalin. Addition of 10 ml. of glycerine to 100 ml. of 5 per cent formalin prior to heating is sometimes recommended for the roundworms. If the situation is such that helminths cannot be allowed to stand for several hours to allow relaxation prior to fixation, hot water may be used for immediate kill. As a matter of fact this method is preferred in some laboratories. Nematodes may be dropped directly into container with steaming water. Large nematodes should be killed in a container large enough to allow the worms to straighten to their full length. Small trematodes or cestodes may be transferred directly into hot water with aid of eye dropper or forceps. Larger trematodes (1/4 inch or larger) should be flattened between micro slides in flat bottom dish, e.g., Petri dish, prior to introduction of steaming water or hot fixative. Larger containers, e.g., rectangular pyrex dish or stainless steel surgical pans may be necessary for satisfactory handling of the larger cestodes. The tapeworm should be grasped in mid-section with forceps then pulled rapidly, following a zig-zag or a figure eight course in the container with hot water. Relaxation occurs at the instant the parasite is killed by the heated water. The cestode should be cut into convenient lengths or placed in position desired in flat dish or pan before fixative is added. All helminths killed by heated water should be transferred to fixing solution within a few minutes of death to avoid post-mortem alteration of tissues.

Any of several fixatives may be used for helminth parasites. Formaldehyde (5-7 per cent) solution plus glycerin (1-10 per cent) is a good general fixative and one that is readily available. Alcohol (60-70 per cent) plus glycerine is a good fixative and is recommended for killing nematodes. F A A (formalin-acetic acid-alcohol) is an excellent all-round fixing agent recommended for use with all helminths as well as for pathologic tissues containing helminths and their eggs. It is easily prepared:

Formalin - 10 parts; 85 per cent ethyl alcohol - 85 parts; and glacial acetic acid - 5 parts. Keep a stock solution containing the above proportions of alcohol and formalin. Add the acid to measured quantities of this as needed for use. After 8-24 hours of fixation in formalin or FAA, the specimens should be washed in several changes of distilled water or in 60 per cent alcohol respectively, prior to staining or subsequent special handling. Specimens should be placed in 60-70 per cent alcohol plus glycerine if they are to be stored for later study or transmitted to a laboratory for identification.

Preparation of Mounts of Adult Helminths

Temporary Mounts - Almost all adult worms can be identified macroscopically, or microscopically under the dissecting microscope or the low-power objective of a compound microscope. Fresh gravid proglottides of tapeworms and living adult flukes may be pressed between two slides and examined by transmitted or reflected light in the low range of magnification. Immersion in 75 parts carbolic acid and 25 parts xylene will clear the animals and is of value particularly for fixed specimens.

Permanent Mounts - Excellent and striking permanent mounts can be made of many helminths, especially the flukes and tapeworms. The stains usually employed are carmine or hematoxylin solutions. However, each worker prefers his own technic, and these are so detailed and varied that it is impossible to outline them here. A standard text on microscopic technic* should be consulted by one desiring to make such preparations. A very practical and simple method has given excellent results recently at the U.S. Naval Medical School.

Glycerogel Method (Yetwin, 1944)**. This is used for mounting small worms of all groups, larvae and eggs.

Technic - Fifteen gm. Bactogelatin (granular, Difco) are dissolved in 150 ml. boiling water. To this are added 50 ml. glycerine (reagent, Merck). After mixing thoroughly, 100 ml. of a 1 per cent chromium and potassium sulfate solution (C.P., granular, Merck) and 1 ml. liquid phenol (U.S.) are added. The medium jells at room temperature but liquefies when heated. The specimens are transferred directly from formalin or glycerine solutions to the mounting medium and allowed to cool. Slides are ready for study or class use in about 10 hours.

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*Galigher, A.E., 1934. The Essentials of Practical Microtechnique, p.288. Albert E. Galigher, Inc., Berkeley, California

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Preservation of Fecal Specimens

This procedure is used in cases where immediate examination of the stool is not practical. The fecal specimen is diluted with tap water until a thin paste is formed. This mixture and an equal amount of steaming 10 per cent formalin are poured together into another vessel. This is set aside for a few hours. Then the supernatant solution is decanted and the formalin replaced. Note: Formalin-preserved ova are heavier than those from unfixed stools. If flotation methods are used to concentrate them, solutions of 1.20 or higher must be employed.

MIF vial preservation method is highly recommended (page 54).

Preservation of Helminths Recovered from Feces

Ova and larvae - The immature stages of the helminths after concentration by sedimentation or centrifugation may be preserved in steaming 10 per cent formalin. If it is desired to keep the ova for periods longer than about 1 year, they may be transferred gradually through a graded series of alcohols, 30 to 50 to 70 per cent.

Adults - A simple and practical method of fixation which is satisfactory for identification and preservation of laboratory specimens is given above. In cases where fine histologic detail is desired, more complex fixing solutions are required, and a text on microscopic methods should be consulted. The fixative used and technic employed will vary depending upon the species involved.

Examination of Perianal, Perineal, and Other Body Regions

Removal and study of material from around the anus is the method of choice for the diagnosis of Enterobius vermicularis. Only 5 to 10% of those infected with this parasite show ova in the feces. Some workers have recently reported higher positive findings for Taenia saginata, Schistosoma mansoni, and S. japonicum by this technic than when the feces alone are examined. Ascaris and Trichuris ova also may be found by this method, but fecal examination is more efficient for these species. For best results the specimen should be obtained before defecation or bathing in the morning. It is recommended that several specimens be taken on different days before a negative report is given.

NIH Anal Swab

This is used primarily for E. vermicularis, but it is also applicable to the other species listed above. The advantages are the very simple technic combined with high efficiency. The scraping can be done by the patients or their parents, and the specimen sent to the laboratory. However, a certain amount of preparation is required which is unnecessary if the specimens can be taken by the technician and immediately examined.

Technic - The perianal and perineal folds are scraped firmly with the prepared anal swab. The rod is replaced in the tube until ready for examination.

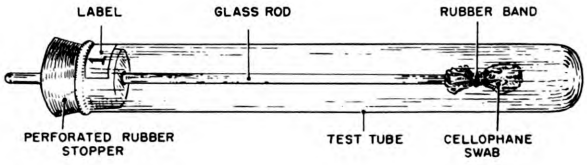

Fig. 91 - National Institute of Health (NIH) anal swab for pinworm

Then the rubber band is cut, and the cellophane is spread on a slide with a few drops of sodium hydroxide or water. This is covered with another slide or a heavy cover glass and examined microscopically.

Scotch Tape Method

This is used for the same species as the NIH swab. It is a very quick and efficient method requiring very simple apparatus. It is not so well adapted for use by the patient with later diagnosis in the laboratory as is the NIH swab.

Technic - A small strip of transparent scotch tape is placed, with the adhesive side out, over the rounded end of a small test tube. The test tube is rocked backward and forward on the perianal folds, allowing the tape to pick up the fecal debris. The tape is then placed, with sticky side down, on a slide and examined microscopically.

Examination of Sputum

Sputum is primarily examined for the ova of Paragonimus westermani, the lung fluke. In a very few cases, however, larvae and adults of Strongyloides and more rarely larvae of Ascaris and the hookworms have been found. In cases where a hydatid cyst of the lung has suppurated or a liver cyst ruptured into the lung, scolices and fragments of the cyst wall may be found in the sputum.

Direct Smear

This is used for all the species listed above, particularly the lung fluke. It is a simple method requiring little apparatus. It does not concentrate the parasites.

Technic - The mouth is rinsed out thoroughly with hydrogen peroxide, and the patient is asked to cough up sputum from the lower respiratory passages into a cup. The sputum is examined microscopically as for fecal specimens, particular attention being paid to iron-brown specks which may be the ova of the lung fluke. In cases where echinococcosis is suspected, Best's carmine is recommended to differentiate the smaller fragments.

Centrifugation

This method is used especially for detection of lung-fluke ova. It gives an effective concentration of parasites. It requires more apparatus and time than does the simple smear.

Technic - If the sputum is at all thick, an equal amount of 3% sodium hydroxide solution is added to it before centrifuging at high speed. After centrifugation, the supernatant solution is decanted and the sediment examined microscopically.

Preservation of Lung-Fluke Ova

Ova of the lung fluke may be preserved in a solution of 5 parts glycerin, 1 part phenol, and 94 parts water.

Examination of Urine

Urine is primarily examined for the ova of Schistosoma haematobium; microfilariae of Wuchereria bancrofti and larvae of Strongyloides stercoralis have also been recovered. Eggs of the dog kidney worm (Dioctophyme renale), a very rare parasite of man, are passed in urine.

Sedimentation

This method is used for the detection of ova of Schistosoma haematobium.

Technic - The technic is the same as for examination of the feces except that no dilution is necessary.

Centrifugation

Centrifugation is useful for the detection of S. haematobium ova, W. bancrofti microfilariae, and S. stercoralis larvae.

Technic - This technic is also the same as that for feces. No dilution or straining is necessary, however.

Hatching Technic

This is used for S. haematobium. The method is the same as for feces. S. haematobium miracidia are evenly distributed in the water, not concentrated at the surface.

Examination of Blood

Blood is examined for the microfilariae of all filarial worms except Onchocerca volvulus. The specimens should be collected at the most advantageous time of the day or night depending upon the species (see laboratory diagnosis of somatic nematode infections). Larvae of Trichinella spiralis occur in the blood from the sixth day after infection until the end of their migration period but can be demonstrated only in very heavy infections.

Fresh Smear

The fresh smear is examined primarily for microfilariae which are easily seen under the low-power objective of the microscope. Microfilariae have a characteristic writhing movement. The method is a simple one requiring little apparatus; it misses light infections and is often not convenient.

Thin Blood Smear

For the detection of parasites, the thin blood smear is made and stained like the thin smear for malaria (page 64). Frequently light infections are missed, and the stain is often not adequate for species differentiation.

Thick Blood Smear

This is made and stained with Giemsa's like the thick smear for malaria. By using a measured quantity of blood, usually 20 cu. mm., it can be made roughly

quantitative. If Wright's stain is used, the smear must be dehemoglobinized. This is a very advantageous technic since both malaria and filariasis can be discovered on a single slide and by using a single stain. It is usually the method of choice.

Concentration

Centrifugation or sedimentation - Centrifugation or sedimentation is used for the detection of microfilariae and the larvae of Trichinella spiralis. Laked blood is usually used for the latter, for which the method is of value only in heavy infections. It affords a concentration of microfilariae and larvae. However, it is time consuming, requires appropriate apparatus, and is seldom necessary since the thick smear will give nearly as good results.

Technic - Ten ml. blood are obtained and citrated (10 ml. blood to 2 ml. 2% sodium citrate in normal saline) or laked (10 ml. blood to 50 to 100 ml. 2% acetic acid or 10/N hydrochloric acid). The citrated or laked blood is centrifuged at 1,000 revolutions for 5 to 10 minutes. The sediment is then removed from the bottom of the tube and examined microscopically, or a smear is made which is dried and stained. Note: Sedimentation for 12 to 24 hours can be substituted for centrifugation if the blood is laked.

Knott concentration - This is used for filarial survey work, especially in areas where the microfilariae show nocturnal periodicity. It is more convenient to take day smears. The method is excellent for survey work and can be made quantitative.

Technic - One ml. blood is mixed with 10 ml. 2% formalin in a centrifuge tube. The mixture is centrifuged at 1,000 revolutions for 5 minutes or sedimented for 16 to 24 hours. The supernatant fluid is decanted, care being taken to get rid of surface bubbles. The sediment is aspirated with a capillary pipet and spread on a slide over areas about 22 mm. square. It is dried in air and fixed for 10 minutes in equal parts of ether and 95% alcohol. The slide is dried in air and stained for 40 to 60 minutes with Delafield's hematoxylin. It is rinsed quickly in 0.05% hydrochloric acid, washed in running water until the blue color appears, and dried in air. A drop of immersion oil is placed on the smear and examination is made systematically with the oil-immersion objective. Some workers prefer to place a cover slip on the preparation.

Special Staining Methods

Although the stains recommended above are usually adequate for finding the microfilariae, they are often inadequate in bringing out the fine detail necessary for identification. The following methods are recommended in these instances and also when good permanent slides are desired:

Vital stains - These are used to differentiate microfilariae of the various species. The method is simple requiring little apparatus; however, the slides are not permanent, and it is often inconvenient to carry out this method of staining them.

Technic - Under a cover slip a very small drop of blood is mounted. A drop of 1 part methylene blue to 5,000 parts physiological saline are drawn under the cover slip, and the slip is ringed with petroleum jelly. If microfilariae are rare, they may be concentrated in several milliliters of blood by dehemoglobinization and centrifugation before staining.

Hematoxylin stains - These are used when fine detail and permanent slides are desired. Bohmer's, Heidenhain's, Delafield's, or other hematoxylin stains may be used. The following method has given excellent results at the Naval Medical School.

Technic - A thick blood smear is made and thoroughly dried. The smear is dehemoglobinized for at least 1 hour during which the water is changed several times. After drying, the slide is passed through or rinsed in the following solutions: methyl or absolute alcohol, 95, 80, 70%, and tap water (3 min. each); Harris' hematoxylin (4 to 8 min.); rinsed in tap water; alcohol 50% (3 min.); acid alcohol until pink; rinsed in 70% alcohol and placed in alkaline alcohol until blue; eosin alcohol (2 min.); rinsed in 95% alcohol and passed into absolute alcohol (3 min.); acetone, xylol, second xylol (5 min. each). Canada balsam is dropped on the blood film, and a cover slip applied before all of the xylol has evaporated, otherwise the field will be milky and ruined. Note: The time in the hematoxylin will vary between 4 and 8 minutes. Both stain and counterstain timing may have to be adjusted. Particular attention should be given to decolorization. Giemsa's stained specimens may be decolorized with acid alcohol and restained as above.

Solutions - Acid alcohol - 2 ml. hydrochloric acid per 100 ml. 70% alcohol by volume. Alkaline alcohol - sodium bicarbonate added to 70% alcohol until the solution turns litmus paper blue. Eosin alcohol - 4% solution of eosin in 95% alcohol.

Examination of Fluid Aspirated from a Hydrocele, Lymph Node, Skin Nodule, or Cyst

Microfilariae of Wuchereria bancrofti and W. malayi may be recovered from fluid taken from a hydrocele or enlarged lymph node; those of Onchocerca can be aspirated from skin nodules. Scolices are demonstrated from a hydatid cyst. The material removed may be studied in fresh smears or centrifuged and the sediment examined. For identification of species of microfilariae, smears are made and stained by dilute Giemsa's or hematoxylin.

Examination of Fluid Removed by Duodenal Drainage

Strongyloides stercoralis larvae and the ova of the liver flukes may be recovered by duodenal drainage. Some workers report increased numbers of positive findings as compared to fecal examinations. However, duodenal drainage is not recommended for the diagnosis of parasitic infection except in cases where all other technics have been tried, and there still remains a definite suspicion that one of the above species is involved. Sediment is obtained by centrifugation or

sedimentation, and examined microscopically.

Examination of Material Obtained at Biopsy (or Autopsy)

The presence of schistosomes, somatic tapeworms, and somatic roundworms which have a more or less prolonged phase in the tissues of man may be diagnosed by examination of biopsy material. Specimens are usually taken from the skin, subcutaneous tissues, lymph nodes, or somatic muscles. The technics given below are also applicable to autopsy material.

Compression Method

This is used for Trichinella spiralis larvae encysted in muscle tissue. Small strips of deltoid, biceps, or gastrocnemius muscle are examined. It is the method of choice for biopsy specimens. It should be combined with digestion for autopsy material. The technic is easy to perform and has a high degree of accuracy. However, it is not adapted to the handling of large amounts of tissue.

Technic - One-gram sections are teased out on a compression slide and examined systematically under a dissection microscope or the low-power objective of a compound microscope. Caution: Care must be exercised not to be misled by old infections.

Digestion Methods

For Trichinella spiralis - In the detection of Trichinella spiralis, the digestion method is used primarily for autopsy specimens in which large amounts of tissue may be examined. It may be used for biopsy material also, but it is not recommended since the compression method is quicker and more accurate for small amounts of tissue. The technic will not detect larvae less than 21 days old; calcified larvae are frequently missed.

Technic - Up to 200 gm. of ground-up diaphragm or other muscle tissue are digested in a liter of digestive fluid (pepsin, 5 gm.; hydrochloric acid, 155 ml.; water, q.s. to 1,000 ml.). This is placed in an incubator at 37° C. for 12 to 18 hours; it is stirred occasionally and allowed to sediment. The supernatant fluid is discarded, and the remainder is screened (20- to 40-mesh) and resedimented twice in water of the same temperature. The sediment is examined under a dissecting microscope.

For Schistosoma mansoni - The digestion method is used for both biopsy and autopsy material. Small bits of the rectal ampullae may be biopsied. Portions of the intestine, liver, and lung may be examined at autopsy. The compression method (see above) is also applicable and is recommended by some workers.

Technic - Small pieces of tissue are digested in 4% potassium hydroxide at 60 to 80° C. for 3 hours. After sedimentation or centrifugation, the sediment is examined with the low-power objective of the compound microscope.

Parasites Teased Out in Normal Saline

This method is used for the cysticercus larvae of Taenia solium removed from the skin, subcutaneous tissues, or superficial musculature; the skin nodules containing Onchocerca volvulus adults and microfilariae; and the adults of Wuchereria species within biopsied lymph nodes.

Fixation, Sectioning, and Staining of Helminths in Tissue

Portions of the biopsied tissue suspected of containing helminths, especially the somatic forms, should be fixed in Zenker's fluid (potassium dichromate, 2.5 gm.; mercuric chloride, 5 to 8 gm.; distilled water, 100 ml.; add 5 ml. glacial acetic acid just before use) and sectioned and stained by the usual pathologic methods.

Recovery of Larvae

Baermann Method

The Baermann method is used especially to recover hookworm and Strongyloides larvae from feces or after culture. It gives excellent results with a high effective concentration. It may also be used after culture to estimate the number of hookworm ova in a given specimen. It is time consuming.

Technic - A short rubber tube is attached to the stem of a large glass funnel (preferably ribbed) and the funnel placed in a ring stand or other like support. The rubber tube is shut off by clamp or other method. A specimen of soil, feces, or culture material is placed in a sieve of bronze or brass screening (1-mm. mesh) which is lined with cheesecloth and made to fit in the funnel. Lukewarm water is poured in the funnel to a height above the lower level of the specimen in the sieve when the latter is placed within the funnel. At the end of 1 to 6 hours, 25 to 50 ml. water are drawn off into a centrifuge tube and centrifuged. The supernatant fluid is pipeted off immediately and the sediment examined microscopically. A piece of ice placed on top of the specimen may speed up the migration of the larvae downward. The larvae may be inactivated by a few drops of 5% sodium hydroxide or iodine solution. Caution: Free-living roundworms in the soil are also concentrated by this method and must be distinguished from the parasitic forms.

Serologic and Allied Tests for Helminthic Diseases

Nearly every important helminthic disease has had a serologic test designed for its diagnosis. These tests, however, are of practical value only for cases in which diagnosis by other methods is impractical at certain stages of the disease and in which the worms cause severe tissue damage, i.e., schistosomiasis, paragonimiasis, echinococcosis, cysticercosis, trichinosis, and filariasis. The tests usually have a group type of reaction and consequently require careful interpretation. Often intradermal, precipitin, and complement fixation tests are available for the same disease, but only the most practical are recommended as follows.

Tests for Schistosomiasis

Complement fixation - The antigen used in this test is an alcoholic extract of the larval stages of the blood flukes in heavily infected molluscan tissues.

The test is of value particularly in early cases before eggs are extruded and in late cases where tissue reaction prevents the passage of ova. It may also be used to diagnose unisexual infections.

Euglobulin precipitation (Sia) - This is not a true serologic test. (See page 88 for description.) It must be carefully evaluated.

Intradermal test - The antigen consists of saline extracts of triturated adult worms or heavily infected snail livers.

The test is of value only in diagnosis since the reaction remains positive after treatment. It is not so effective as the complement fixation test at present but promises to become more useful.

Tests for Paragonimiasis

Complement fixation - The antigen is Paragonimus westermani adults macerated in physiological saline.

The test is of value in cases where eggs are not passed in the sputum or feces.

Tests for Echinococcosis

Intradermal test (Casoni) - The antigen is a clear, sterile hydatid fluid aspirated from the cysts in sheep or other common hosts. Antigen made by the Dennis (1937)*method is probably the most potent.

The test is of value in all cases of echinococcosis but particularly so prior to operation. It is the test of choice in all cases except old complicated ones.

Complement fixation - Purified powdered antigen prepared by Dennis technic is used. This is diluted 1 : 5000 and used as in the Kolmer modification of the Wassermann test.

The test is of value in old complicated cases, but otherwise, the intradermal test is superior.

Tests for Cysticercosis

Intradermal test - The antigen consists of fluid from cysticercus larvae of various species in domestic animals.

*Dennis, E.W., 1937. A stable concentrated purified antigen for the immunological study of hydatid disease. J. Parasitol 23(1):62-67

The test is of value in suspected cases. It should be very carefully evaluated and confirmed by the precipitin test. It is not as reliable as the tests for echinococcosis.

Precipitin test - The same antigen is used as for the intradermal test just described.

The test is of value in confirming a diagnosis of cysticercosis made by the intradermal test.

Tests for Trichinosis

Intradermal test - The antigen is most satisfactorily made from trichina larvae ground in distilled water, dried, and reground.

The test is of value after the twenty-first day of infection. Very heavy and very light infections are often missed. False positive and nonspecific reactions may occur. The reaction remains positive for years after recovery from the acute attacks.

Precipitin test - The same antigen is used as for the intradermal test for trichinosis.

The test is of value after the fourth week of infection. It is of about the same specificity as the intradermal test.

Test for Filariasis (due to Wuchereria species)

Intradermal test - The antigen is prepared most commonly from Dirofilaria immitis, the dog heartworm. Washed, dried, lipoid-free worms are ground into a fine powder. Antigen made by the Bozicevich and Hutter (1944)* method is probably the most potent. Hunter, Bozicevich, and Warren (1945) have shown that antigen prepared from the microfilariae of W. bancrofti is more specific, but it is more difficult to obtain.

The test is of value especially in detection of Wuchereria infection before microfilariae are present in the peripheral blood or when there is a light infection in which the embryos may never be found.

*Bozicevich, J. and Hutter, A.M., 1944. Intradermal and serological tests with Dirofilaria immitis antigen in cases of human filariasis. Am. J. Trop. Med.: 24(3):203-208.

Examination of Intermediate Hosts for Helminth Larvae

Only the examination of the more important intermediate hosts is briefly outlined below.

Snails (for larval trematodes)

Collection of snails - Great care must be exercised in collecting snails in areas where schistosomiasis is present. Since infection may result from contact with a snail from which cercariae are escaping or from contact with water containing cercariae, it is advisable to wear rubber gloves and boots. In areas where this disease is absent these precautions are unnecessary. A small net with a coarse mesh may be used to separate the snails from the mud. Mollusks which are resting in moss may be obtained by shaking small quantities over a pan, screen, or pieces of burlap. Those which are resting on plants may be removed with forceps.

Isolation and identification of cercariae - Snails which are brought into the laboratory should be placed in half-pint bottles, in lots of about a dozen. A gauze top should be placed over the jar to prevent their escape. Each jar should be examined daily for emerged cercariae. It may be desirable to isolate the snails, once cercariae are demonstrated, in order to ascertain the source of cercarial production. Cercariae can be found by crushing the digestive gland located in the spire of the snail. The shells are usually quite delicate, and if care is exercised, they may be cracked without injury to the tissues within. The pieces should be removed and the digestive gland exposed, macerated in water, and examined microscopically. Cercariae obtained in this manner may be immature and unlike those escaping naturally; therefore this procedure should be used only if there is not sufficient time to await the normal emergence of cercariae. Each species of fluke parasitic in man has cercariae which may be identified by their structure. Insofar as possible, cercariae should be examined in the living state with the help of such stains as neutral red or Nile blue sulfate (1 : 1000). Those of the schistosomes are characterized by (1) the presence of a forked tail and (2) the absence of a pharynx; not all forked-tailed cercariae attack man.

Identification of snails - The correct identification of snails is difficult and should be referred to an expert whenever possible. The National Museum* will classify material sent to it and return the identification to the collector. Snails must be handled carefully, relaxed prior to fixation and well preserved if one expects immediate attention and accurate identification by a specialist.

Aquatic snails should be anesthized with nembutal or with menthol crystals. These should be introduced slowly into container of water with snails. Although

*Curator of Molluscs
U. S. National Museum, Washington 25, D.C.

slow and time consuming aquatic species may be suffocated if placed in a capped bottle filled to the top to exclude air. After relaxation the snails may be killed and/or fixed with hot water or hot fixative (70 per cent alcohol plus 2 per cent glycerine). Amphibious and terrestrial snails are easily killed by the suffocation method. All snails should be fixed in a volume of alcohol and glycerine which is at least four times the volume of the specimen. Alcohol should be changed at least one time prior to storage of snails in vials or before transmittal to a museum. Large empty shells should be wrapped in paper and then carefully packed into boxes. Pill boxes lined with cotton serve as an excellent means for collection and safe storage of small shells. Collection data should always accompany specimens: date; locality in which collected; collector; and geographic and ecologic information is of particular importance.

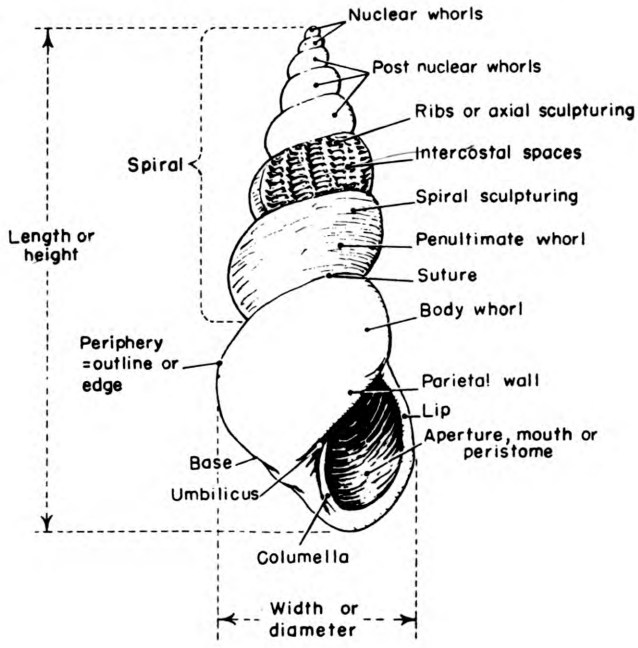

Fig. 92 - Diagram of a snail to show identification characters

Snails can usually be identified by the form and markings of their shells and by the structure of the radula, a lingual ribbon or file-like set of teeth placed in the buccal cavity of each animal. The lingual ribbon is built of thousands of tiny teeth of glass-like substance arranged in closely packed rows. Each row has seven teeth: one central tooth, or rachidian, bordered on both sides by three different teeth - the lateral or median, the inner marginal, and the outer marginal.

Preparation of radula for microscopic examination - The radula is heated gently in strong potassium hydroxide and the flesh needled away. It is then washed in water, stained in mercurochrome, and transferred to 90, then 98% alcohol, then xylol. It is mounted in Canada balsam on a slide. The teeth are broken apart with needles before the slide cover is applied. Enough variation in denticle counts has shown that radulae are helpful but not final in identification.

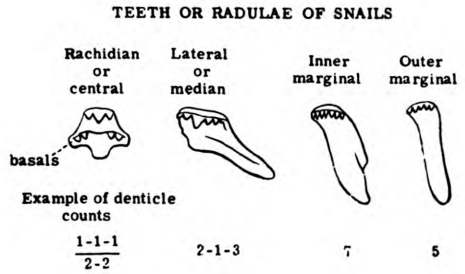

Fig. 93 - Diagram of radulae of snails

Mosquitoes and Other Dipterous Insects (for larvae of filarial worms)**

Technic - Female mosquitoes or other dipterous insects are killed by chloroform, cyanide, or other methods. Identical specimens are identified and preserved or mounted for confirmation. The wings and legs are removed. The abdomen is separated from the head and thorax and placed in separate drops of physiological saline on the same or different slides. The abdomen is teased and macerated with fine needles, and the material is examined for microfilariae under the low-power objective of a compound microscope. The process is repeated with the head and thorax, search being made for advanced stages of larvae. All forms present should be counted and the age of the worms noted. For permanent record, all positive preparations may be fixed with Schaudinn's or other fixative and stained with hematoxylin and eosin.

Food (for infected larval forms of helminths)

The most important foods containing infective stages of helminths are pork and pork products, beef and beef products, fish, crayfish, and vegetables. All of these foods except vegetables may be examined best by the methods discussed under autopsy and biopsy technics, i.e., teasing out the infective form in normal saline, compression, digestion, or sectioning. Pork is examined for trichina larvae and the cysticercus form of Taenia solium. Beef is investigated for the cysticerus of Taenia saginata. Fresh-water fish are inspected for the pleroceroids of the broad fish tapeworm and the metacercariae of certain liver and intestinal flukes; although salt-water fish may in certain cases carry infective forms of some flukes, as a general rule they can be absolved of any relation to helminthic disease in man. Crayfish in endemic areas carry the metacercariae of Paragonimus in their gills or muscles of the appendages.

Vegetables may act as the instrument of conveyance of the metacercariae of the sheep fluke and the giant liver fluke in the rather restricted endemic areas. They have a cosmopolitan importance, however, in helminths transmitted by ingestion of infective ova. Especially in areas where human feces are used as fertilizer, Ascaris, Trichuris, and other ova contaminate the vegetables. They may be removed by scrubbing and recovered by centrifugation or flotation.

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The Navy has specialists in various fields (biology, zoology, entomology, etc.) related to epidemiology, tropical medicine and parasitology. Corpsmen or other personnel in the field should feel free to use the services of these specialists by writing to the Commanding Officer of the Naval Medical School, Bethesda, Md. Frequently these specialists can be of considerable help by lending assistance in identification of insects, mites and venomous animals or may suggest means by which unusual problems in the epidemiology, detection and control of diseases may be approached.

**For technics relating to Entomology reference should be made to "Laboratory Guide to Medical Entomology with a Section on Malaria Control". U.S. Naval Medical School, National Naval Medical Center

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